SHOUT
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BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

Editorial Staff

E. G. Conklin—*The University of Pennsylvania.*
Jacques Loeb—*The University of California.*
T. H. Morgan—*Columbia University.*
W. M. Wheeler—*American Museum of Natural History, New York.*
C. O. Whitman—*The University of Chicago.*
E. B. Wilson—*Columbia University.*

Managing Editor

Frank R. Lillie—*The University of Chicago.*

Volume XVII.

WOODS HOLE, MASS.
JUNE 1909 TO NOVEMBER 1909.

E157(1)
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PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

875

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BIOLOGICAL BULLETIN

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I. TRUSTEES

AUGUST, 1907

EX OFFICIO

C. O. WHITMAN, *Director,* University of Chicago.

F. R. LILLIE, *Assistant Director,* University of Chicago.

E. G. GARDINER, *Clerk of the Corporation,* 131 Mt. Vernon Street, Boston, Mass.

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H. C. Bumpus	American Museum of Natural History, New York City.
D. Blakely Hoar	*Treasurer,* 161 Devonshire Street, Boston, Mass.
W. A. Locy	Northwestern University, Evanston, Ill.
Jacques Loeb	University of California, Berkeley, Cal.
F. P. Mall	Johns Hopkins University, Baltimore, Md.
Gilman A. Drew	University of Maine, Orono, Me.
George T. Moore	Water Mill, N. Y.

TO SERVE UNTIL 1910

E. G. Conklin	University of Pennsylvania, Philadelphia, Pa.
Camillus G. Kidder	27 William Street, New York City.
M. M. Metcalf	Oberlin College, Oberlin, O.
William Patten	Dartmouth College, Hanover, N. H.
D. P. Penhallow	McGill University, Montreal, Canada.
Jacob Reighard	University of Michigan, Ann Arbor, Mich.
W. B. Scott	Princeton University, Princeton, N. J.

TO SERVE UNTIL 1909

S. F. Clark	Williams College, Williamstown, Mass.
Charles Coolidge	Ames Building, Boston, Mass.
C. R. Crane	2559 Michigan Boulevard, Chicago, Ill., *President of the Board.*
T. H. Morgan	Columbia University, New York City.
L. L. Nunn	Telluride, Colo.
John C. Phillips	299 Berkeley Street, Boston, Mass.
Erwin F. Smith	United States Department of Agriculture, Washington, D. C.
E. B. Wilson	Columbia University, New York City.

TO SERVE UNTIL 1908

W. K. Brooks	Johns Hopkins University, Baltimore, Md.
C. W. Hargitt	Syracuse University, Syracuse, N. Y.
C. A. Herter	Bellevue University Medical School, New York City.
H. S. Jennings	Johns Hopkins University, Baltimore, Md.
William Libbey	Princeton University, Princeton, N. J.

A. P. Mathews	University of Chicago, Chicago, Ill.
W. P. Wilson	Philadelphia Commercial Museum, Philadelphia, Pa.

EXECUTIVE COMMITTEE

C. R. Crane,
C. O. Whitman,
F. R. Lillie,
E. G. Gardiner,
E. G. Conklin.
E. B. Wilson,
Jacob Reighard.

TRUSTEES

AUGUST, 1908

EX OFFICIO

Frank R. Lillie, *Director*, University of Chicago.
Gilman A. Drew, *Assistant Director*, University of Maine, Orono, Me.
T. H. Montgomery, Jr., *Clerk of the Corporation*, University of Pennsylvania.

TO SERVE UNTIL 1912

W. K. Brooks	Johns Hopkins University, Baltimore, Md.
C. W. Hargitt	Syracuse University, Syracuse, N. Y.
C. A. Herter	Bellevue University Medical School, New York City.
H. S. Jennings	Johns Hopkins University, Baltimore, Md.
William Libbey	Princeton University, Princeton, N. J.
A. P. Mathews	University of Chicago, Chicago, Ill.
M. J. Greenman	Wistar Institute of Anatomy and Biology, Philadelphia, Pa.
G. H. Parker	Harvard University, New Haven, Conn.

TO SERVE UNTIL 1911

H. C. Bumpus	American Museum of Natural History, New York City.
D. Blakely Hoar	*Treasurer*, 161 Devonshire Street, Boston, Mass.
W. A. Locy	Northwestern University, Evanston, Ill.
Jacques Loeb	University of California, Berkeley, Cal.
F. P. Mall	Johns Hopkins University, Baltimore, Md.
George T. Moore	Water Mill, N. Y.
C. O. Whitman	University of Chicago, Chicago, Ill.

TO SERVE UNTIL 1910

E. G. Conklin .... Princeton University, Princeton, N. J.
Ross G. Harrison ...... Yale University, New Haven, Conn.
Camillus G. Kidder .... 27 William Street, New York City.
M. M. Metcalf .... Oberlin College, Oberlin, O.
William Patten ...... Dartmouth College, Hanover, N. H.
D. P. Penhallow ...... McGill University, Montreal, Canada.
Jacob Reighard .... University of Michigan, Ann Arbor, Mich.
W. B. Scott .... Princeton University, Princeton, N. J.

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C. R. Crane,
C. O. Whitman,
F. R. Lillie,
E. B. Wilson,
E. G. Conklin,
Jacob Reighard.

II. ACT OF INCORPORATION

No. 3170.

COMMONWEALTH OF MASSACHUSETTS

Be It Known, That whereas Alpheus Hyatt, William Sanford Stevens, William T. Sedgwick, Edward G. Gardiner, Susan Minns, Charles Sedgwick Minot, Samuel Wells, William G. Farlow, Anna D. Phillips and B. H. Van Vleck have associated themselves with the intention of forming a Corporation under the name of the Marine Biological Laboratory, for the purpose of establishing and maintaining a laboratory or station for scientific study and investigation, and a school for instruction in biology and natural history, and have complied with the provisions of the statutes of this Commonwealth in such case made and provided, as appears from the certificate of the President, Treasurer and Trustees of said Corporation, duly approved by the Commissioner of Corporations, and recorded in this office:

Now, therefore, I, HENRY B. PIERCE, Secretary of the Commonwealth of Massachusetts, *do hereby certify* that said A. Hyatt, W. S. Stevens, W. T. Sedgwick, E. G. Gardiner, S. Minns, C. S. Minot, S. Wells, W. G. Farlow, A. D. Phillips and B. H. Van Vleck, their associates and successors, are legally organized and established as, and are hereby made, an existing Corporation, under the name of the MARINE BIOLOGICAL LABORATORY, with the powers, rights, and privileges, and subject to the limitations, duties, and restrictions, which by law appertain thereto.

Witness my official signature hereunto subscribed, and the seal of the Commonwealth of Massachusetts hereunto affixed, this twentieth day of March, in the year of our Lord ONE THOUSAND EIGHT HUNDRED AND EIGHTY-EIGHT.

HENRY B. PIERCE,
Secretary of the Commonwealth.

[SEAL.]

III. BY-LAWS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

I. The annual meeting of the members shall be held on the second Tuesday in August, at the Laboratory, in Woods Hole, Mass., at 12 o'clock noon, in each year, and at such meeting the members shall choose by ballot a Treasurer and a Clerk, who shall be, *ex officio,* members of the Board of Trustees, and Trustees as hereinafter provided. At the annual meeting to be held in 1897, not more than twenty-four Trustees shall be chosen, who shall be divided into four classes, to serve one, two, three, and four years, respectively, and thereafter not more than eight Trustees shall be chosen annually for the term of four years. These officers shall hold their respective offices until others are chosen and qualified in their stead. The Director and Assistant Director, who shall be chosen by the Trustees, shall also be Trustees, *ex officio.*

II. Special meetings of the members may be called by the Trustees, to be held in Boston or in Woods Hole at such time and place as may be designated.

III. The Clerk shall give notice of meetings of the members by publication in some daily newspaper published in Boston at least fifteen days before such meeting, and in case of a special meeting the notice shall state the purpose for which it is called.

IV. Twenty-five members shall constitute a quorum at any meeting.

V. The Trustees shall have the control and management of the affairs of the Corporation; they shall present a report of its condition at every annual meeting; they shall elect one of their number President and may choose such other officers and agents as they may think best; they may fix the compensation and define the duties of all the officers and agents; and may remove them, or any of them, except those chosen by the members, at any time; they may fill vacancies occurring in any manner in their own number or in any of the offices. They shall from time to time elect members to the Corporation upon such terms and conditions as they may think best.

VI. Meetings of the Trustees shall be called by the President, or by any two Trustees, and the Secretary shall give notice thereof by written or printed notice sent to each Trustee by mail, postpaid. Seven Trustees shall constitute a quorum for the transaction of business. The Board of Trustees shall have power to choose an Executive Committee from their own number, and to delegate to such Committee such of their own powers as they may deem expedient.

VII. The President shall annually appoint two Trustees, who shall constitute a committee on finance, to examine from time to time the books and accounts of the Treasurer, and to audit his accounts at the close of the year. No investments of the funds of the Corporation shall be made by the Treasurer except approved by the finance committee in writing.

VIII. The consent of every Trustee shall be necessary to a dissolution of the Marine Biological Laboratory. In case of dissolution, the property shall be given to the Boston Society of Natural History, or some similar public institution, on such terms as may then be agreed upon.

IX. These By-Laws may be altered at any meeting of the Trustees, provided that the notice of such meeting shall state that an alteration of the By-Laws will be acted upon.

X. Any member in good standing may vote at any meeting, either in person or by proxy duly executed.

IV. REPORT OF THE TRUSTEES

At the meeting of the trustees held in Woods Hole on August 11, 1908, the resignations of the Director of the Laboratory, Professor C. O. Whitman, and of the Assistant Director, Professor Frank R. Lillie, were presented. The trustees expressed their deep regret that Professor Whitman and Professor Lillie, after so many years of able and efficient services, should wish to resign. The letter of resignation of Professor Whitman, and the reply of the trustees is here given.

To the Trustees of the Marine Biological Laboratory,
Woods Hole, Mass.

Gentlemen: This year has brought the twenty-first birthday of the Marine Biological Laboratory. For these many years you have continued to honor me with the directorship of the laboratory. In late years I have so far drifted out of office and out of use that a formal resignation at this time can be scarcely more than an announcement of the fact accomplished. The time has arrived, however, when a reorganization seems to be imperatively demanded, and as a prelude thereto, I must ask you to accept this note as a somewhat belated announcement of my resignation of the office of director.

Let me take this opportunity to thank you one and all very heartily for the cordial support you have extended to me.

Respectfully,

C. O. Whitman.

Reply to the Above Letter

August 13, 1908.

The corporation and trustees of the Marine Biological Laboratory, in accepting the resignation of the Director, Professor C. O. Whitman, have ordered to be put upon their records and to be forwarded to Doctor Whitman the following minute:

The corporation and trustees desire to express to the retiring director their regret that he finds it necessary to withdraw from the active directorship of the laboratory, and their appreciation of the inestimable value of his services. Since the establishment of the laboratory at Woods Hole twenty-one years ago, he has been continuously its director and he has to a very large extent guided its growth and development. He has stood for the principles of coöperation and independence which have made the laboratory unique in its character and truly national in its reputation and influence. His high ideals and his generous appreciation of the work of others have been an inspiration to the many biologists, who, during these years, have attended the laboratory. These ideals are the most valuable possession of the laboratory.

The corporation and trustees desire that the retiring director may continue to serve the laboratory as honorary director and trustee and that his presence at the laboratory may continue to be an inspiration in the future as in the past.

To the Corporation and Trustees of the Marine Biological
Laboratory, Woods Hole, Mass.

Ladies and Gentlemen: Your action of August 13, in which you express a desire to have me serve the laboratory as "honorary director and trustee" is in itself alone an all-sufficient reward for whatever service I have rendered as director. Your *good will* is the all-important recompense, and no title that you could confer could add to the weight of your approbation. In fact, titles belittle the spirit. Let me have the latter without the former—without title or office of any kind. Please respect this wish and believe me, as ever, a sincere and devoted friend of the laboratory.

Respectfully and cordially,

C. O. Whitman.

Professor Whitman's resignation as director of the Marine Biological Laboratory, after twenty-one years of service in that position, impressively recalls the inestimable value of his services in the establishment and development of this institution. If we have to-day one of the leading marine laboratories of the world, we owe it in large part to him. The interest of almost every member of this board of trustees and of the corporation was enlisted through his efforts, and the splendid influence which the Marine Biological Laboratory has had upon the development of biology in this country is traceable ultimately to him.

His connection with the laboratory began at a time when it had neither permanent home, recognized standing, nor scientific ideals. Some of the leading biologists of this country felt that it could not compete as a research station with the U. S. Fish Commission Station, backed as the latter was by the resources of the government, and that its chief field of usefulness must be as a summer school. Whitman thought otherwise, and by his real greatness as a scientist, his untiring energy and enthusiasm, his splendid ideals and his unfailing faith and courage he made it from the start the principal center in America for biological research.

From start to finish his ideals for the laboratory were these: (1) A national center for research in every department of biology; (2) a laboratory founded upon the coöperation of individuals and institutions; (3) An organization independent in its government and free to follow its natural course of growth and development. For these ideals he has labored consistently and persistently year after year, sometimes with a disregard of present advantage, to be gained by the sacrifice of one or the other of these ideals, which cost him friendships which he highly prized. At one particular crisis he wrote: "If I have made any enemies through unkindness or injustice, I am sincerely sorry for it; but if I have made any because I have stated my conviction on the question before us I can afford to part with all friends who are made enemies for such a cause. Do not think that I seek enemies or value lightly real friends." His faith in the ultimate achievement of these ideals was so great that he chose rather to sacrifice present good than, as he believed, the future welfare of the laboratory; and his plans for the laboratory were so great, while current resources were so small, that he was frequently charged with being impractical. But it is only fair and just to recognize how much was accomplished by adherence to these ideals and to what an extent the spirit and success of the laboratory are due to them.

Woods Hole is indeed a national center for research in several branches, if not in every department, of biology. Whitman had the wisdom to see that biology could progress only as a whole. "The great charm of a biological station," he wrote, "must be the fullness with which it represents the biological system. Its power and efficiency diminish in geometrical ratio with every source of light excluded." To zoölogy, which was the only subject represented at first, he added botany and physiology and he strove to make Woods Hole a center in each of these departmnts. He was one of the first to insist upon adequate provision for experimental work. He was, we believe, the first in this country to plan and plead for a biological farm for the study of problems of heredity and evolution. He desired to make Woods Hole a center for the comparative study of anatomy, pathology and psychology. Some of these lines of work have since been taken up and largely developed elsewhere, but if Whitman could have had the necessary support in his plans they would have been centered at Woods Hole. This need of a national center of research in every department of biology is still before the laboratory as a living issue, and although this grand concept has so far failed of complete realization, who can say how much the laboratory owes to this catholicity of spirit of its director, how much biology as a whole owes to this splendid ideal?

If the laboratory was to be truly national, Professor Whitman believed that it must be founded upon the coöperation of individuals and institutions; no one man nor institution, however great, could accomplish this purpose. He recognized that common ideals must form the basis of such coöperation, and he sought to bring into close connection with the laboratory every person and every institution that shared these ideals with himself. With these ideals, and by means of his own personal charm and scientific abilities, Whitman secured the coöperation of many of the younger biologists of the country. There was thus developed at Woods Hole a center for research work in biology which has had few equals in the history of the world. By his own work, as well as by his appreciation of the really fundamental problems of biology, he has set a very high standard for the scientific work of the laboratory, and by his kindness, sincerity, and generosity he has called forth similar qualities in others, so that it has been characteristic of Woods Hole, as of few other laboratories at home or abroad, that a spirit of genuine coöperation and mutual helpfulness prevails. Who that experienced it can ever forget the inspiration and enthusiasm of those early years of the laboratory? Who of us can forget the cordial appreciation and generous encouragement which we received from Professor Whitman? Some of us feel that we there incurred a debt of gratitude to him which we can never fully repay. Since those early years other laboratories have arisen and other duties have drawn men away from Woods Hole, but the Marine Biological Laboratory never loses its charm for those who have worked there, and this charm will continue as long as the spirit of generous coöperation, which Whitman instilled into it, prevails.

Finally, Professor Whitman stood for the complete autonomy of the laboratory. Although aid might have been had more than once from universities and institutions by surrendering the independence of the laboratory, he steadfastly and consistently refused to do this, even though in doing so he had to face the opposition of almost all the members of the board of trustees and the corporation. There is still a difference of opinion as to the expediency of this stand, but there is probably no question as to the desirability of the autonomy. If the laboratory can obtain endowments such as to provide for its present and future needs and to insure its independence we shall all greatly rejoice, but whether it shall succeed in this aim or not, we are probably all agreed that this much at least of Professor Whitman's ideal must be maintained, viz: that the laboratory must be left free to grow and develop as its own needs and the interests of science demand.

These are the ideals which Professor Whitman has succeeded in making part and parcel of the Marine Biological Laboratory and which we count among our most valuable possessions. To those who measure the success of an institution by the size of its buildings or endowments, his efforts at Woods Hole may seem in large part to have failed, but those who realize that ideals are the motive forces of the world, that life consists not in abundance of possessions but in abundance of service, that science is not paraphernalia but knowledge—these will not fail to recognize the great value of the work Professor Whitman has done for the Marine Biological Laboratory and for the whole science of biology.

The trustees desire to place in the records of the laboratory the following statement:

By the unanimous vote of the trustees the position of director was tendered to Professor Frank R. Lillie. His acceptance of this post has given the trustees the greatest satisfaction. They feel not only that the material future of the laboratory is assured under his management, but also that the high scientific ideals of the laboratory will be maintained.

The trustees wish to take this opportunity to express to Professor Lillie, as they have to Professor Whitman, their recognition of his splendid service in behalf of the laboratory. They are encouraged to believe that the affairs of the laboratory could not have been placed in abler hands. They wish to make public recognition of their appreciation of Professor Lillie's past services to the laboratory and to express to him their confidence in entrusting the future of the laboratory to his care.

The trustees are also able to announce that Professor Gilman A. Drew, who has successfully had in charge for years first the course in zoölogy and later that in embryology, has accepted the position of assistant director. His acceptance of this position is a guarantee that the part of the executive management of the laboratory which falls to his share will be ably conducted.

V. REPORT OF THE TREASURER

SUMMARY OF THE REPORTS OF THE TREASURER OF THE MARINE BIOLOGICAL LABORATORY JANUARY 1, 1907, TO DECEMBER 31, 1908

Receipts

Cash on hand January 1, 1907	$ 1.19	
Income for 1907	15,779.70	
Income for 1908	17,452.18	$33,233.07

Disbursements

Expenses for 1907	$15,638.38	
Expenses for 1908	17,476.25	
Cash on hand December 29, 1908	118.44	$33,233.07

ITEMIZED STATEMENT OF INCOME AND EXPENSES FOR THE YEAR 1907

INCOME

Annual dues	$ 388.00	
Donations	7,000.00	
Miscellaneous	384.61	
Supply Department	5,177.09	
Tuitions	2,830.00	$15,779.70

EXPENSES

Administration	214.60	
Advertising	73.15	
Biological Bulletin	706.51	
Boats and launch	1,632.98	
Chemicals, chemist supplies, etc.	519.14	
Fish trap	81.17	
Instructors' salaries	1,975.00	
Interest	180.50	
Labor	3,063.01	
Periodicals and library supplies	461.16	
Postage	159.40	
Repairs	911.18	
Sundries	2,375.79	
Supply Department	3,284.79	$15,638.38
Surplus		$ 141.32

Itemized Statement of Income and Expenses for the Year 1908

Income

Annual dues		$ 447.00	
Donations		7,401.78	
Fish trap		40.13	
Miscellaneous:			
Interest on deposits	$ 30.80		
Bath House	48.00		
Mess	121.00	199.80	
Supply Department		6,630.13	
Tuitions		2,733.34	$17,452.18

Expenses

Administration	$ 150.00	
Advertising	128.05	
Bath Houses	70.32	
Biological Bulletin	592.67	
Boats and launch	747.71	
Chemicals, chemist supplies, etc.	752.20	
Instructors' salaries	2,400.00	
Insurance	688.54	
Interest	150.00	
Labor	3,333.30	
Periodicals and library supplies	612.53	
Postage	141.32	
Repairs	739.41	
Sundries	2,289.82	
Supply Department	4,680.38	$17,476.25
Deficit		$ 24.07

MARINE BIOLOGICAL LABORATORY INVESTMENTS

JANUARY 1, 1909

Reserve fund (previously called Endowment Fund):		
Amount of fund January 1, 1907	$ 937.49	
Income to January 1, 1909	324.00	$ 1,261.49
Reserve fund now consists of the following:		
$3,000 Am. Tel. & Tel. Co. 4s, cost	2,921.25	
6 shs. Am. Smelting & Refining Co. Pfd., cost	732.00	
Cash	608.24	
	4,261.49	
The above stocks and bonds are held as collateral for loan of $3,000	3,000.00	1,261.49

Library Fund

Amount of fund January 1, 1907	1,378.50	
Income to January 1, 1909	126.00	
Sale of rights	.81	1,505.31
The Library Fund now consists of the following:		
3 shs. Am. Tel. & Tel. Co., cost	383.25	
⅘ of $1,000 Am. Tel. & Tel. Co. 4s, cost	779.00	
1 sh. Am. Smelting & Refining Co. Pfd., cost	122.00	
Cash	221.06	1,505.31

Lucretia Crocker Fund

Amount of Fund January 1, 1907	2,906.41	
Sale of rights	.94	
Income after paying students' fees	55.00	2,962.35
The Crocker Fund now consists of the following:		
18 shs. Vermont & Mass. R. R. Co., cost	2,416.50	
1 sh. West End St. Ry. Co., cost	83.00	
1 sh. Am. Tel. & Tel. Co., cost	127.75	
⅕ of $1,000 Am. Tel. & Tel. Co. 4s, cost	194.75	
Cash	140.35	2,962.35

Donations not Previously Acknowledged

Mr. C. R. Crane	11,000.00
Mrs. Frank R. Lillie	3,401.78

VI. THE DIRECTOR'S REPORT

I. INTRODUCTION

To the Trustees of the Marine Biological Laboratory.

Gentlemen: I beg leave to submit herewith a report concerning the work of the Marine Biological Laboratory for the years 1907-1908, with an appendix containing a compilation of the publications which have been based, wholly or in part, on work done at the Laboratory during the entire period of its existence. In this introductory statement I shall comment briefly on the general functions of the Laboratory and on each of the main divisions of the report. Acknowledgments are due the treasurer and the librarian for their contributions to the report.

The year 1908 was marked by the resignation of Professor Whitman who had served the laboratory as director from its birth to its coming of age. It has rarely happened that the spirit and ideals of an institution have approximated so closely to the conceptions of its director. No greater tribute could be paid to the wisdom and forethought of the ideals with which Professor Whitman endowed the laboratory at its start than the fact that they have proved the fertile germ of the ideals to which, after twenty-one years of trial, we still remain loyal. Through the organization of the Marine Biological Laboratory Professor Whitman has impressed his high conceptions of scientific investigation upon biology in America, and has thus contributed more than any other single person to the present high status of biological investigation in this country.

The continuation of the work of the Laboratory so that it shall maintain a high standing and continue as an effective factor in the progress of biological investigation imposes no light responsibility upon the trustees and officers. Our purpose is essentially ideal and its pursuit demands our best efforts and our loyalty. I believe that we should continue to maintain the coöperative relations of research and instruction that have always given our laboratory a special character; that we should cultivate the coöperation of institutions of learning and of naturalists from all parts of America, and that we should maintain our democratic form of organization. In the membership of our corporation and of our board of trustees we have a large proportion of the best representatives of biological research. Their continued loyalty to the organization and purposes of the Laboratory will inevitably secure its success; our organization and ideals must therefore be such as to commend themselves to our large constituency.

Trustees.—During the past year the following new members have been added to the board of trustees: Dr. Milton J. Greenman, director of the Wistar Institute of Anatomy and Biology of Philadelphia; Professor G. H. Parker, of Harvard University; Professor Ross G. Harrison, of Yale University, and Professor T. H. Montgomery, Jr., of the University of Pennsylvania. The best thanks of the board of trustees is due these gentlemen for their willingness to serve, and the Laboratory is to be congratulated on the increase in prestige and usefulness assured by their presence on the board. We have to record with profound regret and sorrow the loss by death of the distinguished professor of zoölogy of Johns Hopkins University, William Keith Brooks, who has been a member of the board since 1893.

Staff.—At the close of the season of 1907 Professor Drew resigned as head of the Department of Zoölogical Instruction and his place was filled most ably in 1908 by Professor W. C. Curtis, of the University of Missouri, whose previous long connection with the Laboratory fitted him unusually well to take up this important work. Professor Drew took charge of the instruction in embryology in 1908. Both of these gentlemen will continue in charge of their departments. Professor Drew has also acted as assistant director since last August at the request of the director, and his formal appointment to this office is recommended. Professor A. P. Mathews continued in charge of physiology, and Dr. George T. Moore in charge of botany; special acknowledgments are due both for rendering their services without compensation.

Subscribing Institutions.—The number of subscribing institutions which provide for the fees of students or rental of research rooms was sixteen in 1907 and seventeen in 1908; Rochester University, the University of Cincinnati, Vassar Brothers' Institute, and the U. S. Department of Agriculture dropped out, and McGill University, Pennsylvania State School of Forestry, Williams College, the Wistar Institute of Anatomy and Biology and Yale University were added in 1908. The Wistar Institute subscribed for five rooms and permitted the director of the laboratory to nominate occupants, thus providing for one of our greatest needs. The extension of the system of coöperating institutions is greatly to be desired, and there can be no doubt that it is possible.

Attendance.—The total attendance was 107 in 1907 and 100 in 1908; of this number 60 were investigators in 1907 and 52 in 1908. The principal loss in attendance in recent years has been due to a decrease in the number of the class of beginning investigators; a fact to be regretted because it is from this class that fruitful investigators are to be recruited. The number of independent investigators has decreased but little, being 50 in 1907 and 46 in 1908, two less in physiology and in botany and the same number in zoölogy. It is entirely improbable that the slight decrease in attendance represents any permanent defection, for the character of the support of the laboratory has, if anything, increased in quality rather than diminished. And the growing tendency for biologists to establish summer homes of their own in the immediate neighborhood of the Laboratory is a sign and assurance of stability that outweighs any slight fluctuation of numbers. Nevertheless, it is to be hoped that everything possible may be done to increase the attendance of young investigators, for one of the most valuable functions of the laboratory in the past has been to stimulate the desire and ambition for research.

The number of students in 1908 was one more than in 1907, 48 and 47 respectively. The addition of new courses in 1908 did not bring the expected increase in attendance, but it is hoped that it may do so in 1909. So large an attendance of students as used to come to Woods Hole is hardly to be expected, in view of the more considerable competition of summer courses in universities and other laboratories. Indeed a large increase of students is not to be desired, though we could well care for some more.

Treasurer's Report.—The report of the treasurer again shows an income sufficient for our expenditures, if not for our needs. And again it will be seen that a very large proportion of income is in the form of donations, for the major portion of which we are indebted to the president of the board. Mr. Crane's sympathetic and continual generosity puts us under a load of obligation which we can repay only by devotion of our time and best endeavors to the interests of the laboratory.

Our four main sources of income are: fees for tuition, rental of rooms, sale of biological material and donations. The last is the measure of our deficit, and it is to be questioned if the latter can be reduced relative to the sources of income proper. There is a continual tendency for increase in expense of maintenance due to higher salaries for the greater efficiency that comes of long service, the increasing complexity of means of investigation and the growing cost of everything. We can hardly expect a great increase in tuition fees, though there should be some improvement there. But by our united endeavors we can surely increase the number of subscriptions for rental of rooms. Many rooms are now given free, a policy that has been of great service both to individual investigators and to the Laboratory. It is not proper, nor should we propose, to put the burden of room rents on the shoulders of investigators, but institutions can and should provide for the facilities used by members of their staffs. The cash receipts of the supply department have increased from $2,578.85 in 1900 to $5,616.54 in 1906, and $6,630.13 in 1908. We are far from having reached the limit of possibilities in this direction, and a steady growth is to be expected.

Research Seminar and Evening Lectures.—The research seminar and evening lectures have been continued; they have been well attended, and their influence has been good. The physiology lectures under Dr. Mathews' direction have been a great attraction; a large number of investigators have taken part and the discussions have been lively and stimulating.

Publication of the evening lectures ceased some years ago when the publishers refused longer to bear the deficit. While it is hardly probable that the sale of volumes of the evening lectures can be made to pay the expense of publication, owing to the fact that they appeal for the most part to professional biologists, it should nevertheless be possible to issue them at relatively slight net expense. There can be no question that they filled a decided want. They formed an important element in the general prestige of the laboratory and gave expression to the forms of investigation characteristic of the time and place better than anything else. It is therefore desirable that their publication should be resumed, and this is recommended.

Library.—Miss Clapp, who has served as librarian for many years without compensation and with entire devotion to the interests of the Laboratory, has retired; Dr. Knower, of Johns Hopkins University, is recommended as her successor. While there is a useful collection of books in the library, much remains to be done, and it is to be hoped that considerable additions may be made without much cost by exchange and solicitation of donations of proceedings of learned societies. One of the most important special needs of the Laboratory is a fireproof building and endowment for library purposes. It would add greatly to the attractiveness of Woods Hole, and would facilitate the work of every investigator.

Corporation.—The membership of the corporation should receive the earnest attention of the trustees. Under our democratic organization it is the court of last resort. Its membership should therefore be as representative as possible and no pains should be spared to secure desirable members, and give them reason for real interest in laboratory affairs.

International Zoölogical Congress.—The season of 1907 is memorable in our annals for the visit of the Seventh International Zoölogical Congress to Woods Hole. Some foreign members of the congress came early to America to avail themselves of the opportunity for work at the Marine Biological Laboratory. Others made a relatively long visit. The official visit of the congress was made on August 25, 1907. Fifty members came down by special invitation on the preceding evening and were entertained by the Forbes family at Naushon, by the Bureau of

Fisheries, and by members of the Laboratory in their homes. The balance of the members came next morning, and all were greeted by Professor Whitman for the Marine Biological Laboratory and Doctor Sumner for the Bureau of Fisheries. Inspection of the laboratories followed and lunch was served at the Mess. In the afternoon the U. S. Fish Commission steamer, "Fish Hawk," carried the party to New Bedford, where they embarked for New York.

A special feature of the occasion was the signing of a greeting to the director of the Zoölogical Station of Naples. The names signed to this greeting (see p. 45) constitute the only roster of attendance at Woods Hole.

Publications of the Laboratory.—A research institution with a resident staff has a ready record of its achievements in the publications of its members. But a large part of the achievements of an institution such as ours consists in somewhat elusive influences, and it is difficult to estimate the concrete achievements in the way of publications because in many cases only a part of each research which is undertaken at the laboratory is actually accomplished there. In many cases it is the chief part; in other cases only a secondary part. It is perhaps for this reason that the only report on publications from the laboratory is a partial one made in 1895. In preparing the list of publications from the Marine Biological Laboratory (see p. 57) which forms part of the director's report, all those who have worked at the Laboratory were requested to furnish a list of their publications based wholly or in part on work accomplished at the Marine Biological Laboratory. The labor of compilation has been a considerable one, and is only approximately complete. However, the great majority of investigators have been most kind in furnishing lists of their own publications and it is believed that the list will give a more adequate conception than anything else of the activities of the Laboratory in research.

2. THE STAFF, 1907

C. O. WHITMAN, Director,
Professor of Zoölogy, University of Chicago.

F. R. LILLIE, Assistant Director,
Associate Professor of Embryology, University of Chicago.

ZOOLOGY

I. INVESTIGATION

E. G. Conklin	Professor of Zoölogy, University of Pennsylvania.
C. W. Hargitt	Professor of Zoölogy, Syracuse University.
George Lefevre	Professor of Zoölogy, University of Missouri.
Warren H. Lewis	Associate Professor of Anatomy, Johns Hopkins University.
Frank R. Lillie	Associate Professor of Embryology, University of Chicago.
T. H. Morgan	Professor of Experimental Zoölogy, Columbia University.
C. O. Whitman	Professor of Zoölogy, University of Chicago.
E. B. Wilson	Professor of Zoölogy, Columbia University.

II. INSTRUCTION

Gilman A. Drew	Professor of Biology, University of Maine.
R. A. Budington	Instructor in Biology, Wesleyan University, Conn.
Otto C. Glaser	Instructor in Zoölogy, University of Michigan.
Lorande L. Woodruff	Instructor in Biology, Williams College.
Paul M. Rea	Professor of Biology, College of Charleston, and Director of the Charleston Museum.
Max Morse	Tutor in Natural History, College of the City of New York.

COMPARATIVE PHYSIOLOGY

Albert P. Mathews	Professor of Physiological Chemistry, University of Chicago.
R. S. Lillie	Instructor in Physiological Zoölogy, University of Pennsylvania.
A. J. Carlson	Assistant Professor of Physiology, University of Chicago.
Edward G. Spaulding	Assistant Professor of Philosophy, Princeton University.
Oliver P. Terry	Instructor in Physiology, Purdue University.
Horatio H. Newman	Instructor in Zoölogy, University of Michigan.

BOTANY

George T. Moore	Washington, D. C.
George R. Lyman	Assistant Professor of Botany, Dartmouth College.
Ivey Foreman Lewis	Fellow in Botany, Johns Hopkins University.

Cornelia M. Clapp, Librarian.
Oliver S. Strong, Chemist.
Marion C. Gile, Assistant Librarian.
K. Hayashi, Artist.
Kenji Toda, Artist.

G. M. Gray, Curator of Supply Department.
John Veeder, Cockswain.

THE STAFF, 1908

C. O. WHITMAN, Director,
Professor of Zoölogy, University of Chicago.

F. R. LILLIE, Assistant Director,
Professor of Embryology, University of Chicago.

ZOOLOGY

I. INVESTIGATION

E. G. Conklin	Professor of Zoölogy, University of Pennsylvania.
C. W. Hargitt	Professor of Zoölogy, Syracuse University.
Ross G. Harrison	Professor of Comparative Anatomy, Yale University. (Absent in 1908.)
George Lefevre	Professor of Zoölogy, University of Missouri.
Warren H. Lewis	Associate Professor of Anatomy, Johns Hopkins University.
Frank R. Lillie	Professor of Embryology, University of Chicago.
T. H. Morgan	Professor of Experimental Zoölogy, Columbia University.
C. O. Whitman	Professor of Zoölogy, University of Chicago.
E. B. Wilson	Professor of Zoölogy, Columbia University.

II. INSTRUCTION

Winterton C. Curtis	Professor of Zoölogy, University of Missouri.
Lorande L. Woodruff	Instructor in Biology, Yale University.
Paul M. Rea	Professor of Biology, College of Charleston, and Director of the Charleston Museum.
Webster Chester	Associate Professor of Biology, Colby College.

Edward E. Wildman	Fellow in Zoölogy, Princeton University.
John W. Scott	Westport High School, Kansas City.
A. S. Pearse	Instructor in Zoölogy, University of Michigan.

EMBRYOLOGY

Gilman A. Drew	Professor of Biology, University of Maine.
Lorande L. Woodruff	Instructor in Biology, Yale University.
William E. Kellicott	Professor of Biology, Woman's College of Baltimore.
Harvey E. Jordan	Adjunct Professor of Anatomy, University of Virginia.

PHYSIOLOGY

Albert P. Mathews	Professor of Physiological Chemistry, University of Chicago.
E. P. Lyon	Professor of Physiology, University of St. Louis.
R. S. Lillie	Instructor in Comparative Physiology, University of Pennsylvania.
Edward G. Spaulding	Assistant Professor of Philosophy, Princeton University.

BOTANY

George T. Moore	Water Mill, New York.
John M. Coulter	Professor of Botany, University of Chicago.
B. M. Duggar	Professor of Plant Physiology, Cornell University.
Henry Kraemer	Professor of Botany, Philadelphia College of Pharmacy.
Hermann von Schrenk	Pathologist, Missouri Botanical Garden.
Erwin F. Smith	In charge of Laboratory of Plant Pathology, United States Department of Agriculture.
M. B. Thomas	Professor of Botany, Wabash College.
George R. Lyman	Assistant Professor of Botany, Dartmouth College.
C. J. Chamberlain	Assistant Professor of Botany, University of Chicago.

R. R. Gates	Assistant in Botany, University of Chicago.
C. H. Shattuck	Assistant in Botany, University of Chicago.
W. R. Maxon	Assistant Curator, U. S. National Museum.
Arthur W. Taylor	Principal High School, Hampstead, N. H., Assistant Collector.
Jacob Schramm	Wabash College, Assistant Collector.

Cornelia M. Clapp, Librarian.
Oliver S. Strong, Chemist.
Kenji Toda, Artist.

G. M. Gray, Curator of Supply Department.
John Veeder, Cockswain.

3. SUBSCRIBING INSTITUTIONS, 1907

Academy of Natural Sciences, Philadelphia.
Mount Holyoke College.
Rochester University.
Smith College.
Syracuse University.
University of Chicago.
Columbia University.
University of Pennsylvania.
University of Cincinnati.
Vassar College.
Wellesley College.
Woman's College of Baltimore.
Kansas University Woman's Table Supported by Mrs. Robinson.
Vassar Brothers' Institute.
University of Michigan, Bryant Walker Scholarship.
United States Department of Agriculture.

SUBSCRIBING INSTITUTIONS, 1908

Academy of Natural Sciences, Philadelphia.

Columbia University.

Kansas University Woman's Table Supported by Mrs. Robinson.

Lucretia Crocker Scholarship, Boston Public Schools.

McGill University.

Mount Holyoke College.

Pennsylvania State School of Forestry.

Smith College.

Syracuse University.

University of Chicago.

University of Michigan.

University of Pennsylvania.

Vassar College.

Wellesley College.

Williams College, Walker Scholarship Fund.

Wistar Institute of Anatomy and Biology.

Woman's College of Baltimore.

Yale University.

4. INVESTIGATORS AND STUDENTS, 1907-1908

INVESTIGATORS

1907

a. OCCUPYING PRIVATE ROOMS

1. ZOOLOGY

BONNEVIE, KRISTINE E. H., Konservator, University of Kristiania, Norway.

BUDINGTON, R. A., Instructor in Biology, Wesleyan University, Middletown, Conn.

CHILD, CHARLES M., Assistant Professor of Zoölogy, University of Chicago, Chicago, Ill.

CONKLIN, EDWIN G., Professor of Zoölogy, University of Pennsylvania, Philadelphia, Pa.

COOKE, ELIZABETH, Kearsage Village, New Hampshire.

DICKERSON, MARY C., Providence, Rhode Island.

DOWNING, ELLIOT ROWLAND, Northern State Normal School, Marquette, Michigan.

DREW, GILMAN A., Professor of Biology, University of Maine, Orono, Maine.

GARDINER, EDWARD G., 131 Mt. Vernon Street, Boston, Mass.

GLASER, O. C., Instructor in Zoölogy, University of Michigan, Ann Arbor, Michigan.

VON GRAFF, LUDWIG, Professor of Zöology and Anatomy, K. k. Universität, Graz, Austria.

GUYER, M. F., Professor of Zoölogy, University of Cincinnati.

HEATH, HAROLD, Professor of Invertebrate Zoölogy, Stanford University, Palo Alto, California.

LEFEVRE, GEORGE, Professor of Zoölogy, University of Missouri, Columbia, Missouri.

LEWIS, WARREN H., Associate Professor of Anatomy, Johns Hopkins University, Baltimore, Md.

LILLIE, FRANK R., Professor of Embryology, University of Chicago, Chicago, Illinois.

LOEB, LEO, Associate Professor of Experimental Pathology, University of Pennsylvania, Philadelphia, Pa.

MAAS, OTTO, University of Munich, Munich, Germany.

MAST, SAMUEL OTTMAN, Professor of Biological Sciences, Hope College.

MORGAN, THOMAS H., Professor of Experimental Zoölogy, Columbia University, New York City.

MORSE, MAX, Tutor in Natural History, College of the City of New York, New York City.

MURBACH, LOUIS, Head of Department of Biology, Central High School, Detroit, Michigan.

NOGUCHI, HIDEYO, Associate Professor, Rockefeller Institute for Medical Research.

REA, PAUL M., Professor of Biology, College of Charleston and Director of Charleston Museum, Charleston, S. C.

SCOTT, JOHN W., Instructor in Biology, Westport High School, Kansas City, Missouri.

STREETER, GEORGE L., Associate Professor of Neurology, Wistar Institute of Anatomy, Philadelphia, Pa.

STRONG, O. S., Instructor in Normal Histology and Embryology, Columbia University, New York City.

TINGLE, J. BISHOP, Professor of Chemistry, McMaster University, Toronto, Canada.

WHITMAN, C. O., Professor of Zoölogy, University of Chicago, Chicago.

WILHELME, FELIUS, Stazione Zoologica, Naples, Italy.

WILSON, EDMUND B., Professor of Zoölogy, Columbia University, N. Y.

WOODRUFF, LORANDE L., Instructor in Biology, Williams College, Williamstown, Mass.

2. PHYSIOLOGY

CARLSON, A. J., Assistant Professor of Physiology, University of Chicago, Chicago, Ill.

LILLIE, RALPH S., Instructor in Physiological Zoölogy, University of Pennsylvania, Philadelphia, Pa.

MATHEWS, ALBERT P., Professor of Physiological Chemistry, University of Chicago, Chicago, Ill.

MEEK, WALTER J., Professor of Biology, Penn College, Oskaloosa, Iowa.

NEWMAN, HORATIO H., Instructor in Zoölogy, University of Michigan, Ann Arbor, Mich.

PACKARD, WALES H., Assistant Professor of Biology, Bradley Polytechnic Institute, Peoria, Ill.

SPAULDING, EDWARD G., Assistant Professor of Philosophy, Princeton University, Princeton, N. J.

TERRY, OLIVER P., Professor of Physiology, Indiana Medical College, West Lafayette, Ind.

3. BOTANY

Kellerman, Karl F., Department of Agriculture, Washington, D. C.

Lewis, Ivey Foreman, Fellow in Botany, Johns Hopkins University.

Lyman, George Richard, Assistant Professor of Botany, Dartmouth College, Hanover, N. H.

MacRae, Lillian J., Teacher, South Boston High School, South Boston, Mass.

Moore, George T., West Chester, Pa.

Snow, Julia Warner, Associate Professor of Botany, Smith College.

Stickney, Malcolm E., Assistant Professor of Botany, Denison University.

Smith, Dr. Erwin F., Department of Agriculture, Washington, D. C.

Quirk, Agnes Johanna, U. S. Department of Agriculture, Washington, D. C.

Hedges, Florence, U. S. Department of Agriculture, Washington, D. C.

b. OCCUPYING TABLES

1. ZOOLOGY

Beckwith, Cora J., Instructor in Biology, Vassar College, Poughkeepsie, N. Y.

Browne, Ethel Nicholson, Student, Columbia University, New York City.

Cook, Margaret Harris, Fellow in Biology, University of Pennsylvania, Philadelphia, Pa.

Goldfarb, A. J., Graduate Student, Columbia University, New York City.

Kimura, Tokenzo, Student, Columbia University, New York City.

Morgulis, Sergius, Fellow in Zoölogy, Columbia University, New York City.

Shorey, Marion L., Fellow in Zoölogy, University of Chicago, Chicago, Ill.

Tompkins, Elizabeth M., Teacher, High School, Poughkeepsie, N. Y.

Young, Robert T., Instructor in Biology, University of North Dakota.

2. PHYSIOLOGY

Bunzel, Herbert H., Assistant in Physiological Chemistry, University of Chicago, Chicago, Ill.

STUDENTS

1907

1. ZOOLOGY

1. Bacon, Charles Melville, Beloit College.
2. Beckwith, Angie Maria, Vassar College.

3. Bickel, Mary S., Smith College.
4. Bishop, Mabel, Wellesley College.
5. Bissell, Harold I., Rochester, N. Y.
6. Box, Cora May, University of Cincinnati.
7. Carr, Gloria W., University of Missouri.
8. Chidester, Floyd Earle, Syracuse University.
9. Dowell, Anita Shemwell, Woman's College of Baltimore.
10. Frazee, Mary Louise, Woman's College of Baltimore.
11. Furst, Walter Benedict, Penn. State College.
12. Haines, George Clark, Beloit College.
13. Hallock, Frances A., New York Normal College.
14. Hoge, Mildred A., Woman's College of Baltimore.
15. Johnson, Arthur W., Beloit College.
16. Kite, George Lester, Princeton University.
17. Leahy, George V., St. John's Seminary, Brighton, Mass.
18. Lyon, Mary B., Mt. Holyoke College.
19. Marshall, Margaret E., University of Texas.
20. Merriam, Charles G., Lexington, Mass.
21. Packard, Charles, Syracuse University.
22. Pafford, Howard Thomas, Williams College.
23. Palmer, Samuel Copeland, Swarthmore Preparatory School.
24. Paret, Annie Elizabeth, Miss Hill's School for Girls, Philadelphia.
25. Perkins, Millicent E., Mt. Holyoke College.
26. Rand, Carl Wheeler, Williams College.
27. Sadler, Nettie M., Syracuse High School.
28. Shull, Aaron Franklin, University of Michigan.
29. Spencer, Henry James, Syracuse University.
30. Spooner, Georgina B., Vassar College.
31. Stewart, Norman H., University of Rochester.
32. Strongman, Elizabeth J., Girls' High School, Roslindale.
33. Taylor, Helen Grey, Vassar College.
34. Whitman, Frank W., Hyde Park High School, Chicago.

2. BOTANY

1. Bunton, Lillian E., University of Kansas.
2. Bushee, Grace Lydia, Smith College.
3. Jackson, Ethel Alice, Mt. Holyoke College.
4. Mudge, Cora Bell, Bowditch School, Boston.
5. Reynolds, Carrie, Lake View High School, Chicago.
6. Sachett, Clark Harold, Oberlin College.
7. Thomas, Mason B., Wabash College.
8. Weston, Marion D., Mt. Holyoke College.
9. Wood, Elizabeth M., Dorchester High School, Boston.

3. PHYSIOLOGY

1. Hubbard, Ralph Hustace, Columbia University.
2. Patten, Jane Boit, Simmons College.
3. Tinkham, Florence L., Mt. Holyoke College.
4. Wieman, Harry Lewis, University of Cincinnati.

INVESTIGATORS

1908

a. OCCUPYING PRIVATE ROOMS

1. ZOOLOGY

Budington, Robert A., Associate Professor of Zoölogy, Oberlin College.

Chester, Webster, Associate Professor of Biology, Colby College.

Conklin, Edwin G., Professor of Zoölogy, Princeton University.

Cooke, Elizabeth, University of Pennsylvania.

Curtis, Winterton C., Professor of Zoölogy, University of Missouri.

Drew, Gilman A., Professor of Biology, University of Maine.

Evans, Herbert McLean, Assistant in Anatomy, Johns Hopkins University.

Glaser, O. C., Assistant Professor of Zoölogy, University of Michigan.

Goldfarb, A. J., Graduate Student, Columbia University.

Hegner, Robert W., University of Michigan.

Hooker, Davenport, Graduate Student, Yale University.

Jordan, Harvey E., Adjunct Professor of Anatomy, University of Virginia.

Kellicott, Wm. E., Professor of Biology, Woman's College, Baltimore, Md.

Knower, H. McE., Associate in Anatomy, Johns Hopkins University.

Lewis, Warren H., Associate Professor of Anatomy, Johns Hopkins University.

Lillie, Frank R., Professor of Embryology, University of Chicago.

Loeb, Leo, Professor of Pathology, University of Pennsylvania.

Montgomery, T. H., Jr., Professor of Zoölogy, University of Pennsylvania.

Morgan, T. H., Professor of Experimental Zoölogy, Columbia University.

Morrill, Charles V., Lecturer in Histology and Cytology, College of Medicine, Syracuse University.

Murbach, Louis, Central High School, Detroit, Mich.

Pearse, Arthur S., Instructor in Zoölogy, University of Michigan.

Rea, Paul M., Professor of Biology, College of Charleston and Director of the Charleston Museum.

Riddle, Oscar, Instructor in Experimental Therapeutics and Zoölogy, University of Chicago.

Scott, John W., Professor of Biology, Westport High School, Kansas City, Mo.

Stockard, Charles R., Instructor in Comparative Anatomy, Cornell University Medical School.

Strong, Oliver S., Instructor in Normal Histology and Embryology, College of Physicians and Surgeons, New York City.

Wallace, Louise B., Instructor in Zoölogy, Mount Holyoke College.

Wildman, Edward E., Fellow in Zoölogy, Princeton University.

Wilson, E. B., Professor of Zoölogy, Columbia University.

Woodruff, L. L., Instructor in Biology, Yale University.

Yerkes, Robert M., Professor of Comparative Psychology, Harvard University.

2. PHYSIOLOGY

Knowlton, Frank P., Professor of Physiology, Syracuse University.

Lillie, R. S., Instructor in Comparative Physiology, University of Pennsylvania.

Lyon, Elias P., Professor of Physiology, St. Louis University Medical School.

Mathews, Albert P., Professor of Physiological Chemistry, University of Chicago.

Spaulding, Edward G., Assistant Professor of Philosophy, Princeton University.

Shacklee, A. O., Instructor in Pharmacology, St. Louis University.

3. BOTANY

Chamberlain, C. J., Assistant Professor of Botany, University of Chicago.

Duggar, B. M., Professor of Plant Physiology, Cornell University.

Gates, Reginald R., Assistant in Botany, University of Chicago.

Lyman, George R., Assistant Professor of Botany, Dartmouth College.

Maxon, W. R., Assistant Curator, U. S. National Museum.

Moore, George T., Water Mill, New York.

Shattuck, C. H., Assistant in Botany, University of Chicago.

Thomas, Mason B., Professor of Botany, Wabash College.

b. OCCUPYING TABLES

Bartelmez, George W., Laboratory Assistant, University of Chicago.

Beckwith, Cora J., Instructor in Biology, Vassar College.

Brown, Ethel N., Student, Columbia University.

McKibben, Paul S., Student, University of Chicago.

Morgulis, Sergius, Student, Harvard University.

Spooner, Georgina B., Student, Columbia University.

STUDENTS

ZOOLOGY

1. Alton, Benjamin H., South Dakota State College, Brookings, S. D.
2. Carver, Gail C., Mercer University, Macon, Georgia.

3. Cattell, Eleth, Garrison-on-Hudson, New York.
4. Cattell, McKeen, Garrison-on-Hudson, New York.
5. Clark, Mary A., Mount Holyoke College.
6. Dawson, Ava B., Teacher, Boston Public Schools.
7. Eaton, Elon H., Hobart College.
8. Gardner, Julia A., Johns Hopkins University.
9. Halsted, Harbeck, Western Reserve University.
10. Howland, Ruth B., Syracuse University.
11. Kelly, Frank J., University of Pennsylvania.
12. Kelly, James P., Teacher, Public Schools, New York City.
13. Linton, Eleanor A., Smith College, Northampton, Mass.
14. Mellen, Ida M., Brooklyn, New York.
15. Miller, Newton, Clark University, Worcester, Mass.
16. Noyes, Alice A., Mount Holyoke College.
17. Richards, Esther L., Mount Holyoke College.
18. Roe, Adah B., Woman's College of Baltimore.
19. Sayre, Mary L., Woman's College of Baltimore.

EMBRYOLOGY

1. Beckwith, Cora J., Vassar College.
2. Bullard, Florence L., Vassar College.
3. Chidester, Floyd E., University of Chicago.
4. Day, Clarence M., Wesleyan University.
5. Dean, Rollin C., Wesleyan University.
6. Hayden, Margaret A., Raleigh, N. C.
7. Jackson, Clarence M., University of Missouri.
8. Laurens, Henry, College of Charleston.
9. Lyon, Mary B., Mount Holyoke College.
10. Moody, Julia E., Mount Holyoke College.
11. Rand, Carl Wheeler, Williams College.
12. Shaw, Harold C., Wesleyan University.
13. Spencer, Henry J., Columbia University.
14. Stephenson, Joseph C., University of Chicago.
15. Hewitt, Julia A. W., Wellesley College.

BOTANY

1. Blakey, Eleanor, University of Kansas.
2. Davis, Florence R., Wellesley College.
3. Elting, Rebekah, Vassar College.
4. Hedges, Florence, Bureau of Plant Industry, U. S. Department of Agriculture.
5. Illick, Joseph, Pennsylvania State School of Forestry.
6. Kennedy, Mary E., Mount Holyoke College.
7. Moody, Rebecca E., University of Kansas.

8. Palliser, Helen L., Barnard College.
9. Schafheitlin, Gertrude, McGill University.
10. Schramm, Jacob R., Wabash College.
11. Taylor, Arthur W., Dartmouth College.

PHYSIOLOGY

1. Thayer, Lucy C., Teacher, High School, Medfield, Mass.
2. Mackenzie, Mary D., Western College for Women, Oxford, Ohio.
3. Ullmann, Henry J., University of Chicago.

5. TABULAR VIEW OF ATTENDANCE

1907

INVESTIGATORS—Total		60
Occupying Rooms		
Zoölogy	32	
Physiology	8	
Botany	10	
Occupying Tables		
Zoölogy	9	
Physiology	1	
Botany	—	
STUDENTS—Total		47
Course in Zoölogy	34	
Course in Physiology	4	
Course in Botany	9	
UNIVERSITIES AND COLLEGES REPRESENTED		47
By investigators	26	
By students	21	
SCHOOLS AND ACADEMIES REPRESENTED		13
By investigators	4	
By students	9	

1908

INVESTIGATORS—Total		52
Occupying Rooms		
Zoölogy	32	
Physiology	6	
Botany	8	
Occupying Tables		
Zoölogy	6	
STUDENTS—Total		48
Zoölogy	19	
Embryology	15	
Physiology	3	
Botany	11	

Universities and Colleges Represented		51
By investigators	25	
By students	26	
Schools Represented		5
By investigators	2	
By students	3	

6. RESEARCH SEMINAR AND EVENING LECTURES, 1907-1908

RESEARCH SEMINAR

1907

A. P. Mathews	"Spontaneous Respiration of the Sugars"	July 9
Max Morse	"Reactions of the Meal Worm"	July 9
A. J. Goldfarb	"Study of the Effects of Lecithin on Growth"	July 16
Gilman A. Drew	"The Rate of Egg Production by Domestic Fowls"	July 16
A. J. Carlson	"Some Points in the Secretion of Saliva in Mammals"	July 23
C. M. Child	"Amitosis as a Factor in Normal and Regulatory Growth"	July 30
O. C. Glaser	"A Rapid Method of Demonstrating Habit Formation"	July 30
J. B. Tingle	"E. Fischer's More Recent Work on Proteids"	August. 6

EVENING LECTURES

1907

Wm. Lord Smith	"Tiger Lands"	July 12
E. G. Conklin	"Micro-Photography by Ultra-Violet Light"	July 19
C. M. Child	"Some Consideration on the Physiology of Form-Regulation"	July 26
Frederick V. Coville	"A New Branch of Applied Botany"	August 2
W. B. Scott	"Observations in South Africa"	August 7
A. G. Mayer	"The Role of Magnesium in the Control of Rhythmical Pulsation"	August 9
Wm. Bateson	Demonstrations of Mendelian Inheritance	August 12

Wm. Bateson	"More Complex Phenomena and Morbid Inheritance in Man"	August 13
E. B. Wilson	Demonstrations with lantern slides of photographs of chromosomes	August 15
A. A. W. Hubrecht	"Phylogeny of the Allantois"	August 16

RESEARCH SEMINAR

1908

A. P. Mathews	"The Spontaneous Oxidation of Some Cell Constituents"	July 8
C. R. Stockard	"Studies on the Rate of Regenerative Growth"	July 15
Leo Loeb	"Experimental Production of Decidua"	July 22
A. S. Pearse	"Reactions of Amphibians to Light"	July 29
W. C. Curtis	"Artificial Propagation of Fresh Water Mussels"	August 5

SEMINAR IN PHYSIOLOGY

1908

E. B. Wilson	"Inheritance of Sex"	July 7
E. G. Conklin	"Organization of the Egg"	July 9
R. R. Gates	"Chromosomes in Œnothera"	July 11
T. H. Morgan	"Mendelian Inheritance"	July 14
A. P. Mathews	"Inheritance from the Physiological Standpoint"	July 16
E. G. Spaulding	"Is the Organism a Mechanism?"	July 18
A. P. Mathews	"The Chemistry of Fertilization"	July 21
R. S. Lillie	"Irritability"	July 23
R. S. Lillie	"Contractility"	July 25
A. P. Mathews	"Chemistry of Respiration"	July 28
J. R. Murlin	"Proteid Metabolism of Development"	July 30
J. R. Murlin	"General Metabolism of Development"	July 31
E. P. Lyon	"Tropisms"	August 1
S. O. Mast	"Reactions to Light"	August 4

E. G. Spaulding	"Postulates and Results in Treating the Problem of Behavior"	August 6
G. H. Parker	"Evolution of the Nervous System"	August 8
E. B. Meigs	"Theory of Muscular Contractility"	August 10
E. G. Spaulding	"Biology and Pragmatism"	August 11

EVENING LECTURES

1908

Ulric Dahlgren	"The Electric Fishes"	July 3
Frank M. Chapman	"The Pelicans of America"	July 10
Abbott H. Thayer	"Demonstration of Protective Coloration"	July 10
G. H. Parker	"The Origin of Vertebrate Eyes"	July 24
Theobald Smith	"The Transmission of Immunity from Mother to Offspring"	July 28
M. J. Greenman	"A Sketch of the Wistar Institute and its Work"	July 31
A. G. Mayer	"Effects of Chemicals upon Pulsation"	August 7

7. MEETING OF THE SEVENTH INTERNATIONAL ZOOLOGICAL CONGRESS AT WOODS HOLE

The Seventh International Zoölogical Congress visited the laboratories of the Marine Biological Laboratory and of the U. S. Bureau of Fisheries, at Woods Hole, August 25, 1907 (see page 22). The signatures to the following message to the director of the Naples Zoölogical Station constitute a nearly complete roster of the attendance on this occasion:

Dear Dr. Dohrn:

Members of the International Zoölogical Congress, and the Marine Biological Laboratory and the United States Fish Commission Station, all join in expressing their keen regret for your absence at the meetings of the Congress, in Boston, and at Woods Hole, and the earnest hope that your health and strength may be speedily recovered, and yet many years be added to those you have already given to science and to the development of marine stations.

The message was signed by the following:

C. O. Whitman, Chicago.
Frank R. Lillie, Chicago.
G. Koshewnikov, Moscow.
Ralph S. Lillie, Philadelphia.
W. L. Tower, Chicago.
R. R. Gates, Chicago.
Charles Zeleny, Bloomington, Ind.
Mr. and Mrs. Richard Hertwig, Munich.
O. Maas, Munich.
L. W. van Wijhe.
Edw. G. Gardiner, Boston.
Max Lühe.
Chas. P. Sigerfoos, Minneapolis.
A. J. Goldfarb, Columbia University.

Kristine Bonnevie, Christiania.
Mary C. Dickerson.
O. zur Strassen, Leipzig.
Dr. and Mrs. Ch. Wardell Stiles.
G. Severin, Brussells.
Edith M. Patch.
Henry F. Nachtrieb, Minneapolis.
G. Pigrozzi, Bologna.
P. Revilloid, Geneva.
W. H. Lewis, Baltimore.
O. S. Strong, Columbia University.
Susanna Phelps Gage, Ithaca, N. Y.
Agnes L. Bamberg, Columbia University.
T. Kimura, Columbia University.
Simon Henry Gage, Cornell University.
Chas. W. Hargitt, Syracuse University.
Theobald Smith, Harvard University.
Charles F. Rousselet, London.
Cécile Rousselet, London.
B. H. Ransom, Washington, D. C.
Virginia Ransom, Washington, D. C.
Mrazek, Prague.
Professor R. Blanchard, Paris.
Thomas G. Lee, Minneapolis.
Dr. G. Loisel, Paris.
Mme. G. Loisel, Paris.
Emile Young, Geneva.
D. O. Fuhrmann, Neuchatel.
Dr. G. Horvath, Budapest.
Ch. Gravier, Paris.
Marie Loyez, Paris.
Baron Dubreton, Paris.
Marie Ghigi, Bologna.
George Ghigi, Bologna.
Helen V. Tyrode, Boston.
Marassovich, Russia.
Goette, Strassburg, i E.
v. Haecker, Stuttgart.
Marian H. Mulberger, Stuttgart.
Dr. W. Bergmann, Wiesbaden.
Filippo Cavazza, Bologna.

H. Schauinsland.
C. H. Turner, Augusta, Ga.
E. P. Merian, Basel.
J. Büttikofer, Rotterdam.
Professor D. Stephen Apathy, Kolozsmr, Hungary.
Professor W. Th. Studer, Bern.
Professor D. Emil. A. Goeldi, Bern.
L. Murbach, Detroit.
Dr. Ch. Linder, St. Tuner, Switzerland.
C. M. Clapp, Mt. Holyoke College, South Hadley, Mass.
A. Lawrence Rotch, Boston.
Jas. W. Underwood, Olivet, Mich.
A. Borgert, Bonn.
R. Heymons, Berlin.
Rhumbler.
Dr. W. Blasius, Braunschweig.
C. Zimmer, Breslau.
Byron Horton, Columbia University.
Williams B. Herms.
A. V. Richter, Berlin.
E. M. Wallace, Mt. Holyoke College, South Hadley, Mass.
Emma Longfellow, Mt. Holyoke College.
Sergius Morgulis, Ohio State University.
N. J. Kusnezov, Acad. Sci. St. Petersburg.
S. Metalnikoff, Russia.
J. Versluys, Jr., Amsterdam.
J. Arthur Thomson, Aberdeen.
Lorande Loss Woodruff, Williamstown.
S. Watase, Tokio, Japan.
Herbert Osborn, Columbus, Ohio.
Bernard Hobson, Manchester, England.
Robert A. Budington, Oberlin.
Walter B. Cannon, Harvard Medical School, Boston.
Cornelia Jane Cannon.
George G. Scott, New York.
Edwin G. Conklin, Philadelphia.
Thomas Hunt Morgan, New York.
Lilian V. Morgan, New York.
Theo. D. A. Cockerell, University of Colorado.
Wilmatte Porter Cockerell, Boulder, Col.
Roswell Hill Johnson, Station for Experimental Evolution.

Helen James, St. Paul.
Ross G. Harrison, New Haven.
F. F. Gudernatsch, Czernowitz.
Wm. Patten, Dartmouth College, Hanover, N. H.
Dr. V. Nedrigoilow, Russia.
Professor A. Maximow, St. Petersburg.
Dr. W. Dantchakoff, St. Petersburg.
Dr. N. Samssonow, Russia.
Dr. Wladimiroff, St. Petersburg.
Professor Dr. Eugene Golovine.
K. Derjugin, St. Petersburg.
Edith S. Hoyle, Manchester.
Wm. E. Hoyle, Manchester.

8. MEMBERS OF THE CORPORATION OF THE MARINE BIOLOGICAL LABORATORY

---

1. Life Members

Allis, Mr. Edward Phelps, Jr., Palais Carnoles, Menton, France.

Andrews, Mrs. Gwendolen Foulke, 821 St. Paul St., Baltimore, Md.

Billings, Mr. R. C., 66 Franklin Street, Boston, Mass.

Carey, Mr. Arthur Astor, Fayerweather Street, Boston, Mass.

Clarke, Prof. S. F., Williams College, Williamstown, Mass.

Conklin, Prof. E. G., Princeton University, Princeton, N. J.

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Folsom, Miss Amy, 88 Marlborough Street, Boston, Mass.

Foot, Miss Katharine, 80 Madison Avenue, New York City.

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Gardiner, Miss Eugenia, 15 West Cedar Street, Boston, Mass.

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Hannaman, Mr. Charles E., 103 First Street, Troy, New York.

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Jackson, Mr. Charles C., 24 Congress Street, Boston, Mass.

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Kidder, Mr. Nathaniel T., Milton, Mass.

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Mason, Miss Ida M., 1 Walnut Street, Boston, Mass.
Means, James Howard, 196 Beacon Street, Boston Mass.
Merriman, Mrs. Daniel, Worcester, Mass.
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Minns, Mr. Thomas, 14 Louisburg Square, Boston, Mass.
Minot, Dr. Charles S., Harvard Medical School, Boston, Mass.
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WHITMAN, Dr. C. O., University of Chicago, Chicago, Ill.
WHITNEY, Mr. HENRY M., Brookline, Mass.
WILLCOX, Miss MARY A., Wellesley College, Wellesley, Mass.
WILMATH, Mrs. H. D., Elliott Street, Jamaica Plain, Mass.
WILLIAMS, Mrs. ANNA P., 505 Beacon Street, Boston, Mass.
WILSON, Dr. E. B., Columbia University, New York City.
WILSON, Prof. W. P., Philadelphia Museum, Philadelphia, Pa.

2. MEMBERS 1908

ADAMS, C. F., University of Arkansas, Fayetteville, Arkansas.
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CARLSON, A. J., University of Chicago, Chicago, Ill.
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CHANEY, L. W., Northfield, Minn.
CHESTER, WEBSTER, Colby College, Waterville, Me.
CHILD, C. M., University of Chicago, Chicago, Ill.
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Dorrance, Frances, Dorranceton, Pa.
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Jayne, Mrs. Horace, Wistar Institute of Anatomy and Biology.
Jennings, H. S., University of Pennsylvania, Philadelphia, Pa.
Johnson, J. B., University of Minnesota, Minneapolis, Minn.
Jones, Lynds, Oberlin College, Oberlin, Ohio.
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Loeb, Jacques, University of California, Berkeley, Calif.
Loeb, Leo, University of Pennsylvania, Philadelphia, Pa.
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McClendon, J. S., University of Missouri, Columbia, Mo.
McGregor, J. H., Columbia University, New York City.
Mackenzie, Mary D., Western College for Women, Oxford, Ohio.
McMurrich, J. P., University of Toronto, Canada.
Mall, F. P., Johns Hopkins University, Baltimore, Md.
Mast, S. O., Johns Hopkins University, Baltimore, Md.
Mathews, A. P., University of Chicago, Chicago, Ill.
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Moore, J. Percy, University of Pennsylvania, Philadelphia, Pa.
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Morgan, T. H., Columbia University, New York City.
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Morrill, Charles V., College of Medicine, Syracuse University.
Morse, Max, College of the City of New York.

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Rankin, W. M., Princeton University, N. J.
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Whitman, C. O., University of Chicago, Chicago, Ill.
Wieman, H. L., University of Cincinnati, Ohio.
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Wilcox, Alice, 165 Prospect Street, Providence, R. I.
Willcox, Mary A., Wellesley College, Mass.
Williams, Anna W., 416 West 118th Street, New York City.
Wilson, H. V., University of North Carolina, Chapel Hill, N. C.
Woodruff, L. L., Yale University, New Haven, Conn.
Wright, R. Ramsay, University of Toronto, Canada.
Yerkes, Robert M., Harvard University, Cambridge, Mass.

9. PUBLICATIONS FROM THE MARINE BIOLOGICAL LABORATORY

The following list comprising some 860 titles is a compilation of publications representing the activities of the Laboratory in the line of research from its foundation to the end of the year 1907. While the attempt has been to make the list exhaustive, it is not believed that this result has been attained, because there had been no systematic effort since 1895 to keep account of the publications. The present list includes the list of 1895 published in the eighth annual report, the titles of addresses published in the volumes of lectures delivered at the Laboratory, and the results of a circular letter sent to those who have conducted investigations at the Laboratory from the beginning. The greater number of titles were secured in replies to this circular letter, and the list is therefore official in the sense that it is made up of titles furnished by the investigators themselves. Titles indicated by the letter B are of publications of which only a minor part of the research was accomplished in the Marine Biological Laboratory. A copy of the circular letter referred to above follows:

"I shall consider it a great favor if you will send me a list of your publications which have been based, even in part, on work done at the Marine Biological Laboratory. Will you kindly indicate by the letter *A* publications which have been based on material collected in the main at the Marine Biological Laboratory, and by *B* publications which were not based on such material, but in which part at least of the study was accomplished while a member of the Marine Biological Laboratory. The value of this compilation will depend largely on its completeness.

"Your compliance with this request will be of great service to the Marine Biological Laboratory and its management. Thanking you, I am

"Respectfully yours,
"*Assistant Director, Marine Biological Laboratory.*"

Allabach, Lulu F. Some Points Regarding the Behavior of Metridium. Biol. Bull., X, 1906.

Allen, Bennet M. The Topography of Organs in Typical Segments of Hirudo. Biol. Bull., III, pp. 161–164, 1902.

Andrews, Gwendolen F. The Living Substance as Such and as Organism. Journ. Morph., XII, Suppl., p. 176, 1897.

Atkinson, G. F. The Transformation of Sporophyllary to Vegetative Organs. Biol. Lectures, 1895.

Ayers, Howard. Contribution to the Morphology of the Vertebrate Head. Zool. Anz., 1890.

On the Origin of the Internal Ear and the Functions of the Semicircular Canals and Cochlea. Published by the author, 1890.

Concerning Vertebrate Cephalogenesis. Journ. Morph., IV, 1890.

The Ear of Man: its Past, its Present, and its Future. Biol. Lectures, Marine Biol. Laboratory, 1890.

Die Membrana Tectoria, etc., und die Membrana Basilaris, etc. Anat. Anz., VI, 1891.

Contribution to the Morphology of the Vertebrate Ear. Journ. Morph., VI, 1892.

The Macula Neglecta again. Anat. Anz., VIII, 1893.

Some Nerve Muscle Experiments. Journ. Morph., VIII, 1893.

Ueber das Peripherische Verhalten der Gehörnerven u. d. Wert d. Haarzellen d. Gehörorganes. Anat. Anz., VIII, 1893.

The Auditory or Hair Cells of the Ear, and their Relations to the Auditory Nerve. Journ. Morph., VIII, 1893.

B. Morphology of the Cat; or the M. Flexor Accessorius of the Human and Feline Foot. Science, Sept., 1893.

B. Bdellostoma dombeyi: Study from the Hopkins Marine Labotory. Biol. Lectures, Woods Hole, Mass., 1893.

The Origin and Growth of Brain Cells in the Adult Body. Journ. Comp. Neur., VI, 1896.

B. Methods of Study of the Myxamœbæ and Plasmodia of the Mycetozoa. Journ. App. Micros., I, 1897.

B. On the Membrana Basilaris, the Membrana Tectoria, and the Nerve Endings in the Human Ear. Zool. Bull., I, 1897.

B. Contribution to our Knowledge of the Structure of the Organ of Corti in Man. Scientific Assoc. of the University of Missouri, Jan., 1898.

B. Anatomy of the Myxinoids. I, The Skeleton. Ayers and Jackson. Journ. Morph., 1901, and Bulletin of Univ. of Cincinnati, I.

Bardeen, C. R. On the Physiology of Planaria maculata with Special Reference to the Phenomena of Regeneration. Amer. Journ. Phys., V, 1901.

Essential Factors in the Regeneration of Planaria maculata. Biol. Bull., II, 1901.

The Function of the Brain in Planaria maculata. Amer. Journ. Phys., V, 1901.

Embryonic and Regenerative Development in Planarians. Biol. Bull., III, 1902.

Factors in Heteromorphosis in Planarians. Arch. Entw 'mech., XVI, 1903.

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Vol. XVII. *July, 1909.* *No. 2.*

BIOLOGICAL BULLETIN

KARYOKINETIC FIGURES OF CENTRIFUGED EGGS; AN EXPERIMENTAL TEST OF THE CENTER OF FORCE HYPOTHESIS.

FRANK R. LILLIE.

In the annelid *Chætopterus* the first maturation spindle forms in the unfertilized eggs after they are shed in the sea-water, and develops to the stage of the mesophase (Fig. 1). Here it remains standing without change, it may be for hours, unless the egg be fertilized or otherwise effectively stimulated. The eggs can be obtained at Woods Hole in unlimited amounts for a period of about two months during the summer, and as they may be taken from the worms at any time of day they furnish ideal material. The possibility of experimenting directly on a definite stage of the karyokinetic figure is naturally suggested by the material itself, and I propose to give here an account of the results of experiments with centrifugal force, which have been carried on during four successive seasons, so far as they are related to the karyokinetic figure. A communication of the results was made before the joint session of the Central Branch of the American Society of Zoölogists and Section F (Zoölogy) of the American Association for the Advancement of Science held in Chicago, December, 1907, and an abstract was printed in *Science*, N. S., Volume XXVII., pp. 907–908, June 12, 1908.

The principal advantage of this material, aside from its availability and abundance, is that it offers the rare opportunity of experimenting on a *definite* and *fixed* stage of the karyokinetic figure. At the stage in question the forces concerned in karyokinesis have reached a certain balance which is maintained indefinitely, until the equilibrium is upset by the conditions initiated by entrance of the spermatozoön or some other effective change.

The action of potassium chloride appears to be quite specific in this respect, as was first discovered by Mead ('98), and since described by Loeb ('01) and myself ('02). Such agents set in motion the series of karyokinetic processes, which are then carried through to their completion. But even violent mechanical disturbances, such as are produced by shaking or centrifuging, cause only temporary disturbances, and the equilibrium of the mesophase of karyokinesis is soon reëstablished. The methods by which the regulation, or regeneration, of the karyokinetic figure is brought about after centrifuging appear to me to have some bearing on the problem of the nature of the dynamical complex represented by the karyokinetic figure.

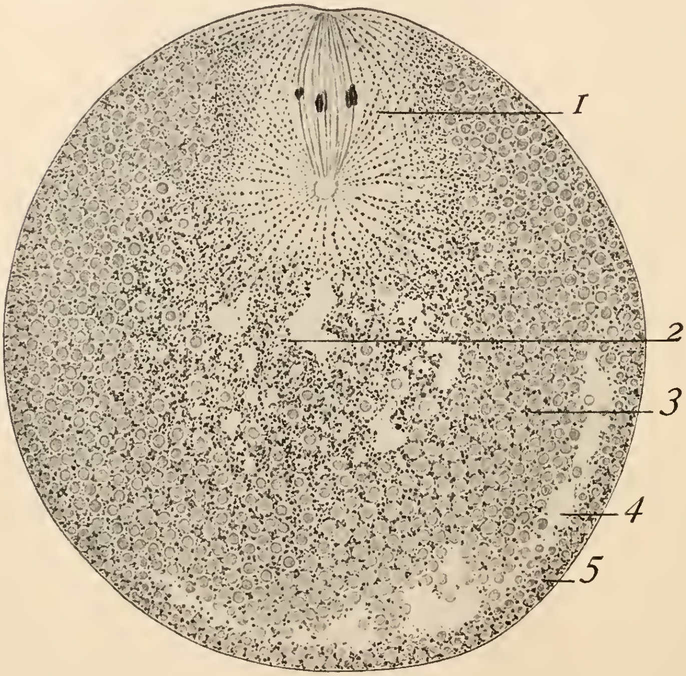


Fig. 1. Section of an egg of *Chætopterus* at the stage used for the experiments. See text for description. 1, karyokinetic figure; 2, central spongy area; 3, inner dense zone; 4, outer spongy area; 5, ectoplasmic layer or outer dense zone.

Fig. 1 shows the typical condition of the eggs before centrifuging. The animal hemisphere contains a large karyokinetic figure at the mesophase with nine chromosomes in the equatorial plane. The outer pole of the spindle is practically in contact with the surface of the egg, which is slightly depressed at the pole. The centrosome of the outer pole is smaller than that of the inner pole. The astral rays cross very extensively opposite the equatorial plane; indeed, rays from the outer pole may extend inward beyond the level of the inner pole, and rays of the latter may extend nearly to the surface of the egg. In the spindle itself, there is the usual distinction between mantle fibers attaching the chromosomes to the two poles and central spindle fibers running from pole to pole. The interfibrillar spaces are perfectly clear. The only asymmetrical feature of the spindle is the somewhat smaller size of the outer pole and the difference in arrangement of the rays at the two poles, owing to the proximity of the outer pole to the surface of the egg.

All eggs show great uniformity, but there may be slight differences between eggs of the same lot in the following respects: (1) In some the outer pole is not actually attached to the surface so as to produce a depression, but may be removed from the surface by a distance of about twice the diameter of the outer centrosome. There is thus a variation in the firmness of fixation of the spindle at the animal pole. (2) There is some variation in the intensity, that is, in the length and strength of the astral rays, which appear less well developed in some cases than in others. In all cases, they cross to a considerable extent in the equatorial plane.

The evidence that is to be presented is furnished by the results of centrifuging the eggs at varying speeds and for various times. To form a proper conception of what happens it is absolutely essential to understand the effects of centrifugal force on the protoplasm. In a previous paper I presented an analysis of this subject and showed that the fundamental structure of the egg as represented by the conformation of the ground substance is not essentially modified by the centrifugal force; it is at most somewhat distorted. On the other hand, the granules suspended in the ground substance move through the latter in a central or distal direction, depending on their relative specific gravities, and arrange themselves in three strata: the stratum nearest the axis of the centrifuge is of course of least specific gravity; it constitutes a cap of granules of intermediate size that give a fatty reaction with osmic acid and are soluble in oils. The intermediate stratum, forming a *hyaline band*, contains very minute granules that take the basic stain; they may therefore be called basophile granules. The distal stratum occupies the distal hemisphere of the egg and is composed of larger granules of yellow tinge, usually called yolk granules, which take the acid stain and may be known collectively as acidophile granules. The chromatin and the spindle have the same specific gravity as the basophile granules, as is proved by the fact that they are found in the hyaline band when they move freely.

The movements of the granules, and consequently their segregation, are modified by differences of resistance within the ground substance. The latter has four concentric zones (Fig. 3, *A*), (1) a central more fluid area (Fig. 3, *A*, 2), (2) a dense zone bounding the latter (Fig. 3, *A*, 3), (3) a second more fluid zone specially developed in the region of the spindle (Fig. 3, *A*, 4), and (4) an ectoplasmic zone defective at the outer end of the spindles (Fig. 3, *A*, 5) (see Lillie '09).

All zones contain both basophile and acidophile granules (Fig. 1). Under the action of centrifugal force, therefore, the granules move relatively freely through the more fluid zones and are impeded in their movements in the denser zones. The consequence is that when low centrifugal speeds of short duration are used, there is a tendency for the granules to aggregate in the dense zones. Thus the basophile granules of the central more fluid area aggregate in the central end of the dense zone bounding it (Fig. 2) and the acidophile granules in the distal end. The basophile granules of the outer fluid zone aggregate proximally with reference to the axis of the centrifuge and the acidophile granules aggregate distally, and therefore lie external to the inner dense zone and within its walls, but do not pass through the latter to enter the central more fluid area (Fig. 2). The most important of these aggregations with reference to the interpretation of the karyokinetic figure is the *basophile cap* found in the central end of the inner dense zone (Fig. 2, *b.c*).

The distortion of the ground substance is of the following character: (1) the egg is invariably somewhat elongated in the line of action of the centrifugal force, owing to the differential force between centripetally and centrifugally moving granules; (2) the principal part of this elongation is born by the massive, external, more fluid zone, which is stretched more on the centripetal side, the consequence being that the inner fluid area and its dense bounding zone lie on the centrifugal side of the middle point of the elongated egg (Figs. 3 and 4).

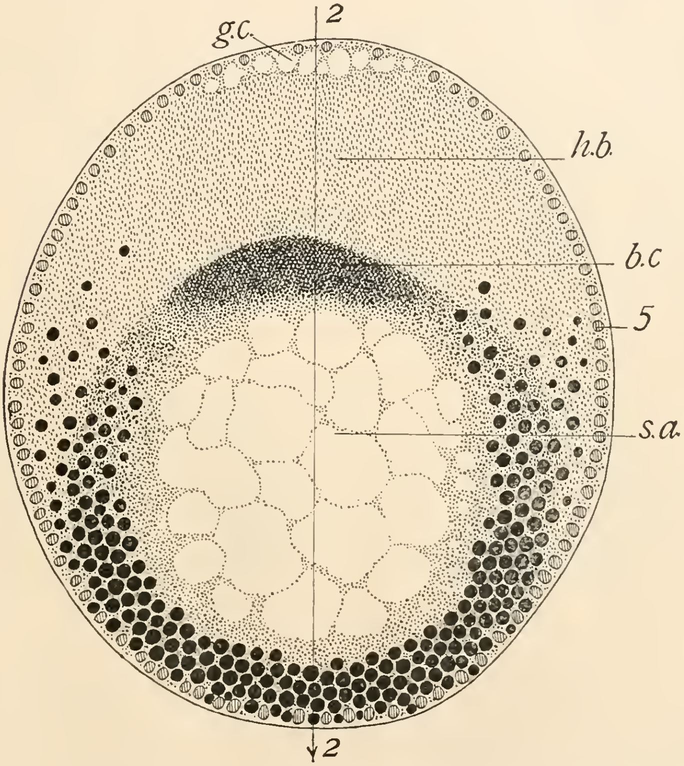
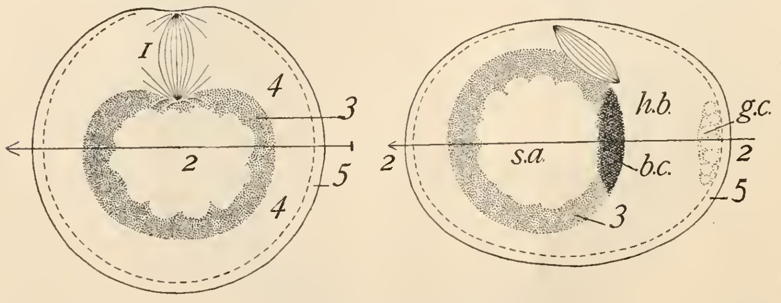


FIG. 2. Section of an egg of *Chætopterus* centrifuged 1,150 revolutions in 31 seconds at a radius of 13 cm. 2–2 indicates the direction of the centrifugal force; 5, ectoplasmic zone; *b.c.*, basophile cap; *g.c.*, gray cap, or fatty granules (dissolved out by the oil used in preparation); *h.b.*, hyaline band; *s.a.*, spongy area. This egg was stained in iron hæmatoxylin and orange G.

The spindle itself may be moved as a result of centrifuging. The simplest cases are those in which the outer end remains fixed and the inner end is swung from its radial position. This shifting appears to be due to distortion of the ground substance with the result that the inner dense zone presses in one or another direction on the spindle and displaces its inner end. In other cases the spindle is moved some distance from the surface into the hyaline band; this result is more commonly found with the higher centrifugal powers.

The normal relation of the spindle to the zones of the ground substance is illustrated in Figs. 3, *A*, and 4, *A*. The inner end of the spindle reaches to the inner dense zone and its antipolar rays are embedded in the latter, which is indented by the spindle. If now the centrifugal force acts parallel to the axis of the egg with the animal pole in a central position, the egg elongates in this axis. If the outer end of the spindle remains attached to the surface, the inner end tends to be withdrawn from the inner dense zone, and the basophile cap formed lies outside of the area of the spindle. If, however, the inner end remains fixed, the outer end is withdrawn from the surface, and the inner end is embedded in the basophile cap.



A *B*

FIG. 3. *A*, diagram of zones of the ground substance in the normal egg and the relations of the spindle. *B*, diagram to illustrate the effect of centrifugal force on the ground substance and spindle acting in the direction of the arrow. 1, karyokinetic figure; 2, central spongy area of control egg; 2–2, direction of action of centrifugal force; 3, inner dense zone; 4, outer more fluid zone; 5, ectoplasmic zone. The numbers have essentially the same significance as in Fig. 1; *b.c.*, basophile cap; *g.c.*, gray cap; *h.b.*, hyaline band.

If the centrifugal force acts at right angles to the axis of the egg, the inner end of the spindle tends to be swung in a proximal direction, (Figs. 3, *A* and *B*) both because the specific gravity of the spindle as a whole carries it in that direction, and also because the contraction of the egg in its own axis brings the dense zone nearer the animal pole and thus exerts pressure on the spindle. Many cases of this kind are found.

If the animal pole is distal in position in the centrifuge the spindle must be subjected to rather violent assaults : first, because its specific gravity tends to drive it towards the opposite end of the egg ; second, because the basophile granules are streaming away from it and the acidophile granules rushing in, and third, because the inner dense zone is approximated to this pole (Fig. 4, *B*). I have not found any eggs with the spindle fixed at the distal pole after centrifuging, and am therefore forced to assume that in all cases in which the centrifugal force acts in this direction the spindle is torn loose from the surface and moves up into the hyaline band.

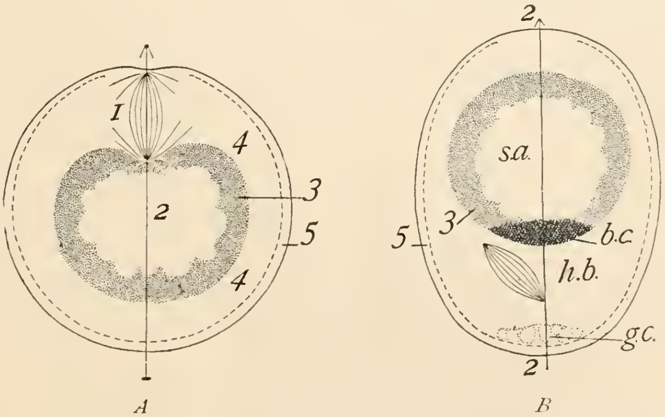


Fig. 4. *A*, diagram of the zones of the ground substance in the normal egg and the relations of the spindle. *B*, diagram to illustrate the effect of centrifugal force on the ground substance and spindle acting in the direction of the spindle. Numbers and letters as in Fig. 3.

Intermediate directions of action of the centrifugal force will affect the protoplasm and the spindle in ways easy to deduce from the above.

If the above analysis of the effects of centrifugal force is correct, or even partially so, we have to distinguish between those cases in which granules are driven out of, or into, the area of the karyokinetic figure, and those cases in which the spindle has moved its position. It is obvious that both conditions may occur simultaneously ; indeed, it is probable that this is almost always the case.

In my paper of 1906, I described and illustrated a naked spindle with centrosomes at its ends, but practically no radiations. This spindle was found in an egg that had been centrifuged about 2,000 revolutions in a minute at a radius of 13 cm., killed in picro-sulphuric acid, sectioned, and stained in Delafield's hæmatoxylin. At the time, I was not paying special attention to the question of analysis of the karyokinetic figure, and I regarded the case as typical.

When I repeated the experiments in the summer of 1906, with special reference to the analysis of the karyokinetic figure, I soon found that the condition of a naked spindle after centrifuging was not typical; on the contrary, it is extremely rare and exceptional. Well-developed asters are the rule after centrifuging, though they may exhibit certain asymmetries or other modifications of the normal condition. Either, then, the rays must persist in spite of movements of the spindle and passage of granules through them, or the naked spindle must have formed new radiations.

Presumably the latter process would require time, and it therefore seemed possible that if special pains were taken to fix the eggs in the shortest possible time after centrifuging, one might find at least a considerable proportion of naked spindles. But this also proved a vain expectation. Typically, astral radiations are well developed after centrifuging; and this is the case no matter how quickly the eggs are killed, nor whether the speed be high or low, within the limits of the actual experiments.

I therefore considered the possibility of fixing the eggs during the action of the centrifugal force, but I did not see clearly how to compass this end and so the experiment was never performed.

I. Movements of the Spindle.

Every case in which it can be demonstrated that the spindle has moved through the cytoplasm furnishes a test between mitome and centrosome hypotheses. If the visible rays are organic radii attached to the poles of the spindle (mitome hypothesis), they must be disarranged by each movement of the latter, and movements of the spindle at different angles to its own axis must produce different characteristic modifications of the rays of the aster. If the spindle moves in the direction of its own axis, for instance, the rays of the forward aster must tend to be bent back about the spindle, and those of the other aster away from the axis. Similarly, there should be definite configurations characteristic of every angle and amplitude of movement. But, if the rays are expressions of forces centered at the poles and are therefore composed of oriented particles (centrosome hypothesis), such disarrangement of the radii would not be expected to result from movements of the spindle, except to the extent involved in the inertia of the substances concerned.

In a considerable number of experiments it was my aim to kill the eggs in the shortest possible time after centrifuging. The tube was removed from the centrifuge as quickly as possible, the water poured off and the killing fluid poured on immediately. Therefore, in two or three seconds after the centrifugal force had ceased to act the eggs were submitted to the action of the killing fluid. Such eggs were sectioned and stained.

The cases that interest us most here are those in which the spindle is found detached from the surface, because it is obvious that such spindles have been moved from their original position : The entire karyokinetic figure in such cases may be perfectly symmetrical if it is found in protoplasm of uniform composition. But differences in the composition of the protoplasm within the area of the karyokinetic figure are correlated with asymmetries of the figure itself which are considered beyond. If the outer pole of the spindle is withdrawn from the surface it is found to possess well-developed antipolar rays, although these were entirely wanting before centrifuging (cf. Fig. 5), and the astral rays are symmetrical around both poles, although "organic radii" would naturally be distorted by such displacement of the "centers of insertion" as is illustrated in Fig. 5. It appears to me that the astral radiations in such cases must be regarded as new. The case is of course analogous to the shifting of sperm asters in fertilization. In the present case, however, we know the time of readjustment to be very short, one minute at the outside, and the initial and reconstructed conditions are before us for comparison (cf. Figs. 1 and 5).

Similar cases, are numerous in eggs that have been submitted to a very strong centrifugal force, 7,500 revolutions in one minute at a radius of 6 cm., because the end effect of such centrifuging is to produce a uniform arrangement of basophile granules in the hyaline band, into which the spindle also is driven. We therefore never find such particomposition spindles as in low powers of the centrifuge (see beyond). The karyokinetic figure is usually symmetrically developed after the strongest centrifuging in spite of the fact that rearrangements of granules are more extensive than with lower centrifugal powers. The reason for this must lie in the uniform character of the protoplasm, which is produced in the hyaline band by strong centrifuging.

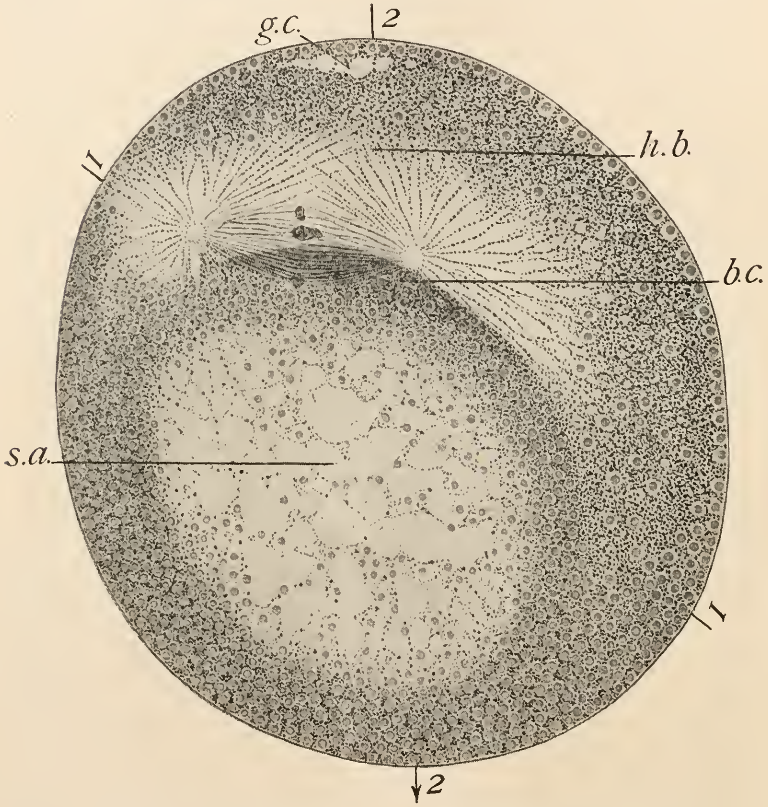


FIG. 5. Section of an egg of *Chætopterus* centrifuged 1,150 revolutions in 31 seconds at a radius of 13 cm. 1–1, primary axis of the egg; 2–2, direction of action of the centrifugal force, secondary axis; *b.c.*, basophile cap; *g.c.*, gray cap; *h.b.*, hyaline band; *s.a.*, spongy area. The egg was stained in thionin and orange G.

A single example of this kind would not be convincing in itself, for it might be maintained that the egg in question deviated originally from the norm of the control eggs. But when such a

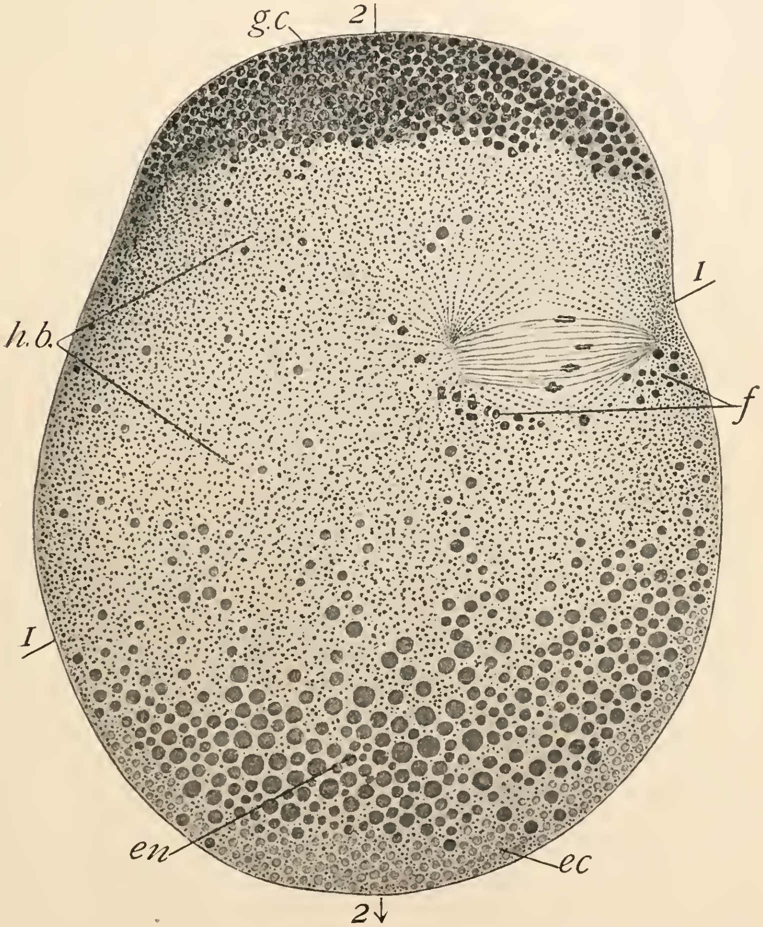


FIG. 6. Section of an egg of *Chætopterus* centrifuged 7,800 revolutions in one minute at a radius of 6 cm. The egg was killed in Flemming's fluid, and the fat granules of the gray cap are stained black. The spindle blocks fat granules passing in a centripetal direction (*f*). 1–1, primary axis of the egg; 2–2, secondary axis; *ec.*, ectoplasmic spherules; *en.*, endoplasmic spherules; *f.*, fat granules blocked by the spindle; *g.c.*, gray cap; *h.b.*, hyaline band.

condition is found to be the type of hundreds of eggs in different experiments with no considerable deviations, as is the case in my experiments, it becomes a conclusive argument for the re-formation of rays in successive positions of the spindle, a condition that can be explained only on the centrosome hypothesis.

Among the numerous cases we find some in which the antipolar rays are very slightly developed at *both* ends; and various intergrading conditions that seem to indicate stages in the formation of new rays.

In the case of eggs submitted to low centrifugal force an occasional karyokinetic figure is found with distorted astral rays. But such a condition must occur as a transition stage in every case of spindle movement on the centrosome hypothesis, because of the mere viscosity of the medium and the inertia of the oriented particles. The occurrence of such conditions is therefore to be expected, and does not furnish any argument against the center of force hypothesis.

2. Movements of Granules into the Area of the Karyokinetic Figure.

The three classes of granules, basophile, acidophile, and fat, may be moved into the area of the karyokinetic figure. As a general proposition we may say that fatty or acidophile granules driven into the area of an aster tend to destroy the radiations and are not themselves arranged in lines. If forced into the spindle itself, they tend to disarrange its fibers. On the other hand, basophile granules that are driven into the area of the asters or spindle do not, apparently, destroy the existing rays or fibers, and are themselves arranged conformably to the lines of the figure.

A. We need not dwell long on the statement concerning fatty and acidophile granules. It is sufficiently supported by Figs. 5 and 6 as regards the asters. No traces of rays are left in invaded areas of the asters except that the scanty basophile granules between the fatty or acidophile granules may show a slight radiate tendency centered at the poles of the spindle. There are no detached rays, no traces of broken down threads; the peripheral ends of rays of invaded areas always disappear absolutely.

Serious disarrangement of the fibers of the spindle is found if fatty or acidophile granules are driven into it. Such cases are relatively rare, but the disarrangement may be much more considerable than in the case illustrated (Fig. 7). The ends of the spindle may be much spread out and the chromosomes may be turned around at right angles to their normal orientation.

B. The effect of the introduction of basophile granules into the area of the karyokinetic figure is, however, entirely different. They cause no disarrangement, but appear to become oriented like the rays of the aster, or the fibers of the spindle. The evidence for this conclusion is based on the behavior of the dense aggregation of the basophile granules found in the central end of the inner dense zone after the action of low centrifugal powers, *i. e.*, of the basophile cap (Figs. 2, 3*B*, and 4*B*).

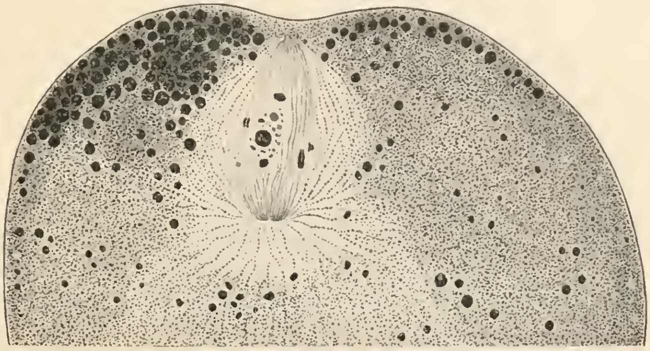


FIG. 7. Section of an egg of *Chætopterus* centrifuged 2,300 revolutions in 70 seconds at a radius of 13 cm. Killed in Flemming's fluid. The figure represents the centripetal half of the egg. Fat granules have entered the spindle and destroyed part of its structure.

The heavy stain of the basophile cap lies entirely in the granules, not at all in the ground substance. It is true that the ground substance between the granules appears darker in the basophile cap than elsewhere, but this is an effect due to the light passing through basophile granules at a lower focus. In thin sections it is not evident at all. The conclusion is really proved by the fact that such an effect is seen only in the basophile cap, and that this may lie in any part of the wall of the dense zone, depending on the direction of action of the centrifugal force.

1. Let us note first that the granules that compose the basophile cap have a uniform distribution when remote from the

spindle (Fig. 2). What happens when they are driven into the area of the karyokinetic figure? A few examples will illustrate:

The egg shown in Fig. 8 was allowed to stand in sea-water about 35 minutes, then centrifuged 1,150 revolutions in 31 seconds at a radius of 13 cm. and killed as rapidly as possible in picro-acetic acid. It was one of a large number. Sections were cut 6 μ thick and stained in iron hæmatoxylin and orange G. All the sections of this egg are present on the slide. The figure represents a single section which includes practically the entire spindle and all nine chromosomes. The plane of the section was nearly transverse to the axis of stratification and is approximately tangential to the basophile cap; but on the right side the section passes nearer to the distal pole than on the left side and therefore takes in a good deal of the distal acidophile stratum on this side, while on the left side the section is confined to the

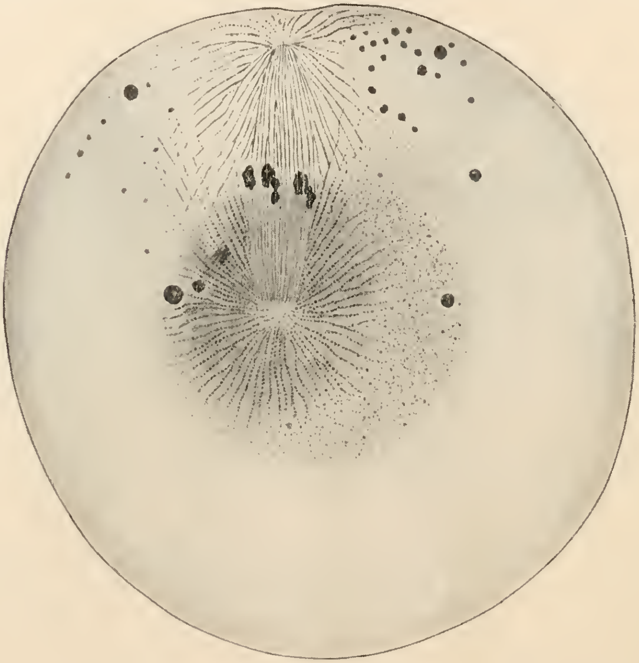


FIG. 8. Section of an egg of *Chætopterus* centrifuged 1,150 revolutions in 31 seconds at a radius of 13 cm. Half of the karyokinetic figure lies in the basophile cap. See text for further description.

hyaline band. The granules of the ectoplasmic layer are apparently unaffected by the centrifugal force. The karyokinetic figure has been moved little, if at all, as is proved by the fact that there is an indentation opposite the outer pole, and by the relation of the ectoplasmic layer which exhibits the characteristic defect around the outer pole of the spindle, which I described in my first paper.

That portion of the karyokinetic figure lying within the basophile cap partakes of its character, and is as sharply differentiated from the remainder of the figure as the basophile cap itself is differentiated from the protoplasm of the hyaline band. As to the nature of these differences: (1) the stain is much darker within the basophile cap; (2) the rays of the aster are more numerous than in the control (Fig. 1) and also more granular; they are in fact obviously made up of linear arrangements of basophile granules; (3) the spindle itself is more darkly stained within the basophile cap than outside of it; this pertains to the fibers themselves, but I would not venture to say that the fibers are more numerous.

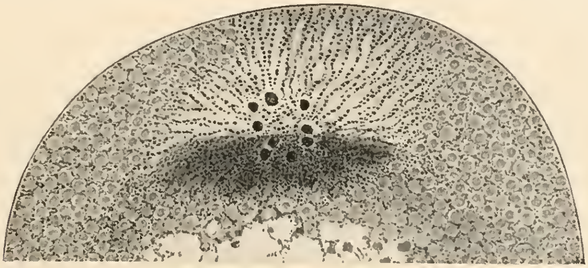


Fig. 9. Transverse section of parti-composition spindle. From section of an egg of *Chætopterus* centrifuged 1,150 revolutions in 31 seconds at a radius of 13 cm. Half of the section of the spindle lies in the basophile cap. See text for further description.

Where a fiber either of the spindle or of the aster crosses the boundary between the basophile cap and the hyaline zone its character changes. Rays of the aster never extend into a compact mass of acidophile granules, hence the present figure is less developed on the right side than on the left.

Fig. 5 represents a spindle placed tangentially to the basophile cap. The contour of the spindle is perfectly regular, but its composition is heterogeneous. For the rest, exactly the same principles apply as in Fig. 8, except that astral rays are not very numerous in the basophile cap; this is due to the fact that the section is near the margin of the cap and contains therefore a large proportion of acidophile granules.

Fig. 9 finally is a transverse section across a spindle lying partly in and partly out of a very dense basophile cap. It is obvious that the spindle area has been invaded on one side by the basophile granules. These are in fact so numerous and are stained so deeply that they almost conceal the chromosomes embedded in them. The concentration of the granules within the spindle area is thus extremely different on the two sides, but on each side it is precisely the same as in the neighboring cytoplasm.

These cases are typical of a large number in my preparations. It is obvious that there is a great difference in the fatty and acidophile granules on the one hand and the basophile granules on the other, with reference to their effect on the karyokinetic figure. The former efface any part of the figure which they occupy, the latter are arranged conformably to the lines of the figure. This difference is not conceivably a mere question of size, it is rather a question of specific behavior. The basophile granules behave as though they were within effective range of centers of force to which they are permeable; the fatty and acidophile granules on the other hand behave as impermeable particles would behave in such a field.

It is worth while to examine this idea more carefully because the center of force hypothesis alone can render account of the results of these experiments. Theoretically, on a center of force hypothesis the following results might be expected: (1) That the number of radiations from the center would be a factor of the density of aggregation of the more permeable particles; if the number of permeable particles within the effective area were greatly increased the number of rays should therefore become greater. (2) The number of oriented particles in the same length of any ray should also be a factor of the density of aggregation of the permeable particles.

We would therefore expect on the center of force hypothesis of the karyokinetic figure that, if the basophile granules are permeable to the force, (1) they would be oriented along the lines of force when driven into the area of the karyokinetic figure; (2) that the number of rays in an unusually dense aggregation of basophile particles would be greater than usual and (3) that the number of oriented particles in each ray in such cases would be greater than usual.

These appear to be the conditions found in my experiments. With reference to the first condition, there can be no question that the basophile granules arrange themselves conformably to the lines of the karyokinetic figure, as already noted. With reference to the number of astral rays one obtains a strong subjective impression that they are more numerous in the basophile cap than in the asters of control eggs, and I have attempted to confirm this by actual counts. To do this it is necessary to divide the aster into sectors for comparison, for the reason that asters lying entirely within the basophile cap do not occur, owing to the form of the basophile cap. I have therefore made comparisons between sectors of 90° of the normal aster and asters of the basophile cap. One has further to restrict the count by limiting it to a single focus of the microscope. Under these conditions I found an average of 8 rays to the 90° sector of the normal aster (eighteen counts) and 12.5 in 90° sectors of asters in the basophile cap (six counts). The difference seems too great to be accounted for by error. The counts confirm the impression that one receives by mere comparison. I do not think that such increase of the number of rays in a sector of the aster could be explained by compression of preëxisting rays from other sectors of the same aster, for they are quite uniformly spaced and are as straight and regular as the normal rays.

As regards the third condition: rays within the basophile cap stain more strongly than those without; but as the stain is held by the granules, this would be evidence for a greater number of granules in the rays. A ray that passes out of the basophile cap loses suddenly in intensity of staining (Fig. 8).

The spindle is decidedly denser than the surrounding protoplasm; this has been noted by several investigators (cf. Foot and Strobell (p. 221), and McClendon). The same thing is shown by some of the phenomena of centrifuged eggs; when, for instance, the direction of centrifugal force is at right angles to the axis of the spindle, granules must stream by it. Now if the density of the spindle area be no more than that of the surrounding ground substance and if there be no repellent force from the spindle, such granules would pass through the spindle readily. But on the contrary they are blocked by the spindle, and heap up against it. Fig. 6 illustrates this condition. The accumulation of granules on the distal side of the spindle is fatty in character, at least the granules are blackened by Flemming's fluid which was used for killing, and they are the same in character as those accumulated at the proximal pole. A pathway of such granules runs from the pole of the spindle towards the proximal accumulation, rendering the interpretation certain. We can readily believe, therefore, that the relatively dense spindle moves as a unit through the protoplasm. If, then, it be thrown into the basophile cap, it will naturally tend to maintain its character; many such cases are in fact found. In other cases, the part of the spindle within the basophile cap is partly changed, partly unchanged; but in very many cases the part within the cap is completely changed, so that the staining reaction of the spindle agrees with that of the cap.

Whether we consider the movements of the karyokinetic figure produced within the egg by centrifuging, or whether we consider the results of driving granules into the area of the karyokinetic figure, the general conclusion that the poles of the spindles are centers of force appears to me to be inevitable; no system of antagonistic fibrillæ could behave in such a way. The difficulties that the centrosome hypothesis has to meet are well known, and their discussion does not enter within the scope of this contribution, which is in a sense a by-product of another problem. I shall therefore be satisfied merely to present the evidence and to indicate the direction in which it appears to point.

In the development of the karyokinetic figure, the rays or fibers appear to grow out of the centers up to the time of the mesophase at least, and this must be due to increasing power of the centers. In a magnetic model on the other hand the orientation of particles along the lines of force takes place simultaneously by segregation along their entire course (Hartog, '05). Now my experiments give conditions quite similar to magnetic models in this respect, for the reason that the centers displaced at the mesophase are in the condition of maximum force.

The fact that the experiments deal only with this stage of the karyokinetic figure limits the results in ways that I fully realize. But this is more than compensated by the advantage of knowing the precise stage of the karyokinetic figure with which the operations deal. The attempts to obtain comparable results at other stages have been unsatisfactory, because any other stage is in motion and the control eggs vary so much that interpretation of results becomes very uncertain.

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THE SPERMATOGENESIS OF AGALENA NÆVIA.

LOUISE B. WALLACE.

INTRODUCTION.

A study of the spermatogenesis of the Araneina offers a field of unusual interest to the investigator not only because of the seemingly aberrant form of the mature sperm cells but also because of the presence of conspicuous accessory chromosomes in many species. One needs only to read over the list of subjects in recent cytological literature to realize how much interest is at present centered upon the development of the germ cells as a whole and especially upon certain chromosomes which behave peculiarly and which are designated by various names — "chromosomes spéciaux" (de Sinéty), "Chromatin nuceolus" and "heterochromosome" (Montgomery), "small chromosome" (Paulmier), "accessory chromosome" (McClung), "idiochromosome," "macrochromosome" and "microchromosome" (Wilson). Nearly two decades ago, Henking ('90) in his work upon *Pyrrhocoris*, discovered and described a chromatin element which took part in only one of the spermatocytic divisions and therefore caused dimorphism of the spermatozoa but he did not apparently see the relation between this element and the chromatin nucleoli of the resting stage nor did he offer any suggestion as to its significance. Since the publication of Henking's work, scores of papers on insect spermatogenesis have appeared but as excellent reviews of this literature have already been given more than once, it seems superfluous to review it here. Suffice it to say that it is now a well-established fact that among the Hemiptera, at least, dimorphism of the spermatozoa is the rule and furthermore that the dimorphism is due to the unequal distribution of the heterochromosomes or to their being of unequal size. In the myriapods, also, Blackman ('01, '03, '05) has found the same dimorphism of the spermatozoa.

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[1] Thesis presented to the faculty of the Graduate School of the University of Pennsylvania in partial fulfilment of the requirements for the degree of Ph.D.

Outside of the insects and myriapods, heterochromosomes have been reported in the Araneina only and as yet this unusually beautiful and interesting material has not only been comparatively neglected, but there has been marked discrepancy in the results of the few who have worked upon it. So far as I know, the only published work upon the development of the male germ cells of spiders which has been done under sufficiently modern methods of technique to be of value to us here is that of Wagner in 1896, myself in 1900, Montgomery, Bösenberg and myself in 1905 and Berry in 1906. With the hope of harmonizing the results of the above-mentioned authors and of correcting and expanding my own earlier results the present work was undertaken. It has seemed best to use the common tube-weaving spider, *Agalena nævia*, again as the basis of investigation, although a number of genera have been studied in order to illustrate some points. While my former conclusions in regard to the behavior of the ordinary chromosomes are now only verified and strengthened, those upon the distribution of the accessory chromosomes and upon the nature of the degenerating cells need considerable revision. After a careful reading of the literature on the subject and after an examination of testes in various genera, I am convinced that before long generalizations can be made in spider spermatogenesis and that contradictions are mainly due to differences in observations and interpretations; not to radical differences existing in the developmental history of the germ cells themselves.

My investigations were carried on in the Zoölogical Laboratory of the University of Pennsylvania and it gives me pleasure to warmly express my indebtedness to Professor Conklin for the kind interest with which he has followed the progress of my work and his helpful suggestions and encouragement.

Material.

Most of my material was collected in the state of Massachusetts where the breeding season of *Agalena* does not begin, usually, until the last week of August and continues until late in September. In mid-summer the testes are largely made up of primary spermatocytes in the growth period while in May and the early part of June they consist mainly of spermatogonia. In Philadelphia the breeding season occurs a week or two earlier than it does in Massachusetts. Late in the fall the male spiders are fewer in number than the female spiders owing to the fact that they are more often overcome and eaten by their mates. Most of the adult spiders perish at the approach of cold weather.

The testes are translucent, slightly convoluted tubular organs and can be easily seen as they lie inbedded in the voluminous, brown liver which in mature specimens occupies the greater part of the abdominal cavity. In very young spiders, however, it is often difficult to distinguish the testes from the whitish, tubular spinning-glands lying beneath them. In *Agalena* it is a comparatively simple matter to determine the sequence of stages in the development of the germ cells as in cross-sections of the testes the least mature cells are always at the periphery, and they increase in maturity toward the lumen. Sometimes in a single cross-section can be found spermatogonia, spermatocytes, spermatids and mature spermatozoa. In the height of the breeding season the lumen and the ducts are filled with quantities of ripe spermatozoa and degenerating cells. As was first discovered by Menge ('43), the male spider has the peculiar habit of spinning a small, delicate web and depositing upon it a minute drop of seminal fluid which is then taken up into the fine, coiled tubes of the pedipalps preparatory to its introduction into the receptaculum seminis of the female. This process can readily be observed if spiders be kept in captivity during the breeding season and Montgomery ('03) has given a detailed description of it in a number of genera. By teasing out the contents of the storing-organs of the pedipalps one can obtain an abundance of spermatozoa which are sure to be mature.

METHOD.

The spiders were beheaded and the testes dissected out in the killing fluid. This method renders easy their removal from the body and insures rapidity of fixation. Among the various fixing fluids used were Zenker's fluid, Gilson's mercuro-nitric, Gilson's acetic alcohyl with sublimate, Hermann's fluid and Flemming's fluid, strong solution. Flemming's fluid gave slightly better results than Hermann's and both of these fluids gave vastly better preparations than the others mentioned although all were useful in some ways.

Ripe spermatozoa from the pedipalps were smeared on a glass slide and well fixed by heating at the boiling point. Smear preparations, after the method of Foot and Strobell, proved of value for the mature sperm cells but were disappointing for the earlier stages. This may be due to the fact that the chromosomes are large and numerous and even when the nucleus is spread out in an extremely thin film, they become so heaped up upon one another that it is not possible to count them accurately. If smear preparations be compared with those treated with fixing fluids, it is evident that the latter cause considerable shrinkage ; and tissues fixed in Flemming's fluid for a few hours show less shrinkage than those fixed for fifty hours or more but the latter method brings out strongly the centrosomes and spindle fibers.

Heidenhain's iron-hæmatoxylin has been chiefly relied upon for staining as it gave the finest results in almost every particular but beautiful preparations were also obtained with Hermann's triple stain. Every one grants that the different reactions to staining reagents are no safe criterion in the endeavor to differentiate nuclear elements, since the same structures do not always stain in the same way during the various phases of development. Nevertheless color-differentiation is often helpful and this is especially true when dealing with the accessory chromosomes. Their whole history can be made out clearly in iron-hæmatoxylin preparations but when Hermann's triple stain is used, their affinity for the safranin at times when the ordinary chromosomes or the chromatin granules take the violet, makes them stand out in a striking manner.

Observations.

Spermatogonia.

In young spiders, early in the summer, the testes are wholly made up of spermatogonia and, unlike most forms, these cells in mitosis are almost equal in size to the spermatocytes of the growth period. In the metaphase the rod-shaped chromosomes are so numerous, probably fifty or more, and so closely packed together, that in polar views it is impossible to make even an approximately accurate count of them (Figs. 1 and 2). It was also not found possible to identify at this stage the accessory chromosomes among the other rod-shaped chromosomes. The centrosomes are quite distinct and the spindle fibers have a tendency to bulge out, forming a spindle with convex sides. This is probably owing to the large size of the chromosomes and to the large number of fibers which must be accommodated between the centrosomes and the equatorial plate. In division the rods split longitudinally and the resultant halves move toward the opposite poles. In anaphase two pairs of daughter chromosomes appear distinct from the others both on account of their large size and because they are slower in passing to the poles (Figs. 3 and 4). While this might well be looked upon as simply a case of retarded division, in the light of what follows it seems probable that they are the accessory chromosomes which have the form of a pair of rods throughout the greater part of their subsequent history. In telophase the ordinary chromosomes gradually become granular but their identity is still traceable in the loose, irregular masses scattered through the nuclear cavity. These masses stain but faintly, and in iron-hæmatoxylin preparations, when the extraction of the dye has not been carried further than usual, they are nearly colorless. The accessory chromosomes, on the other hand, retain the rod-like form and lie near the periphery of the nucleus.

Spermatocytes.

In Fig. 5 is shown the early prophase of the primary spermatocyte in which the chromatin masses are becoming looser, more granular, until finally the processes of adjacent ones meet on the threads of the linin reticulum. The nucleus swells with the accumulation of nuclear sap and at the same time the cytoplasm is reduced to a rather thin layer around the swollen nucleus. The accessory chromosomes lose their rod-like form and appear as two densely staining, chromatin nucleoli often lying at some distance apart (Fig. 6). Later these two chromatin nucleoli approach one another and finally unite to form a single, large, irregular mass which is a conspicuous body in the resting stage (Fig. 7). This is followed by the contraction stage when the whole cell is noticeably reduced in size and the nuclear contents are contracted away from the membrane. At this time the nucleus stains so deeply that only after long extraction can anything be learned of its structure and even then one can merely say that the chromatin appears to be in the form of a dense reticulum. It is apparently at this stage that synapsis occurs, reducing the number of chromosomes to half the spermatogonial number. The large, chromatin nucleolus of the resting stage has again resolved itself into two peripherally placed rods shown in longitudinal and cross sections (Figs. 8 and 9). At the close of the contraction stage the growth period is introduced by an evident increase in the size of the cell and the nucleus. As the nuclear elements become spread out in the enlarging cavity it is comparatively easy to see in what condition they are. The chromatin is now in the form of irregular granules distributed along the delicate loops of the spireme. The loops are long and often twisted or bent, so that their number was not determined nor could I ascertain whether or not the spireme is continuous but taking what evidence one can gather from this stage, together with a study of a slightly later stage, it seems probable that the loops are distinct from each other and that the segments of the spireme are in the reduced number (Figs. 10 and 11). Later the nucleus enlarges still more, allowing the loops to stretch out fully and it is now clear that the spireme is segmented, the free ends of the loops all being directed toward one side of the nucleus (Fig. 12). The linin thread is delicate, at first, and the chromomeres undivided, but later a split runs throughout the length of each loop, dividing the chromomeres equally. Sometimes the latter appear as large after the splitting as before but this is readily explained when one considers that meanwhile the loops have shortened and so have crowded the chromomeres into larger groups. At first glance the accessory chromosomes, on account of their great staining capacity, appear to have maintained their peripheral position but careful focusing reveals the fact that they are now completely surrounded by the spireme loops (Fig. 13). A cross-section of the same stage is shown in Fig. 14. Although the accessory chromosomes have moved to a more central position in the nuclear cavity, their outer ends are still near the membrane and are always directed toward that part of the cell which contains the greatest amount of cytoplasm and in which the centrosome lies. This pole has been called by Montgomery the distal pole. The closed ends of the spireme loops are directed toward the opposite or central pole. The same relative position of the loops, the accessory chromosomes and the centrosome is retained throughout the growth period and gives striking evidence of cell polarity. At a slightly later stage the accessory chromosomes seem to be simulating the structure of the spireme to a limited degree. They temporarily lose their compact form, become distinctly granular and extend in length over about two thirds of the diameter of the nucleus (Fig. 15). Gradually they shorten again and conjugate side by side, the union usually first taking place at the end directed toward the central pole and progressing toward the distal pole (Fig. 16). Cases are found in which the union begins simultaneously at the two extremities, giving a ring-like form. After the union is completed they appear as a single mass when viewed either in longitudinal or cross section (Figs. 17 and 18). Whether in this conjugation a complete fusion of the two elements occurs or whether it is merely a close approximation, I am unable to say, but soon the single mass is again split into the two characteristic rods.

When the longitudinal split in the spireme has reached its widest extent, some of the loops still extend nearly across the nuclear cavity but later they begin to draw down toward the distal pole. As the shortening continues, the longitudinal split becomes less and less evident but indications of it can be detected at a late stage. Judging also from the subsequent history I believe that the split persists, being merely lost sight of in the close approximation of the two moieties during the process of contraction (Figs. 19–21). In the late prophase, the loops of the spireme not only shorten but bend to an acute angle to form V-shaped chromosomes which then open out into double V's (Fig. 22). The chromatin of these is in a more or less granular condition and leaving their former position at the distal pole they are distributed through the nuclear cavity. The rod-like accessory chromosomes remain unchanged both in form and in position. Soon the nuclear membrane disappears, the ordinary chromosomes, having reached their definitive form, become more compact and densely staining and spindle fibers appear (Fig. 23). In metaphase all of the double V's are drawn into the equatorial plate and the plane of division passes through the center of each, giving rise to single V's which are carried to the opposite poles. The accessory chromosomes lie at the periphery of the equatorial plate and are connected by spindle fibers to one pole only, a single fiber passing from the centrosome to each of the two rods. In the succeeding division they pass into but one of the two daughter cells, so that we find half of the secondary spermatocytes with accessory chromosomes and half without them (Figs. 24–26). Those containing the accessory chromosomes might be called "favored cells" ("bevorzugten zellen") as suggested by Henking. In polar views of the telophase several sections were found in which each of the two accessory chromosomes showed a distinct longitudinal split — a precocious splitting which is of interest as foreshadowing the division which occurs in the following mitosis (Fig. 27).

While the arms of the V-shaped chromosomes are elongating and becoming sinuous or twisted in outline, a conspicuous cell-plate forms and the constriction of the cell body grows deeper (Fig. 28). Since the chromosomes are long and twisted at this stage it is plain that even in fairly thin sections there might occur more than one section of a single chromosome and therefore little dependence could be placed upon the number counted in successive sections of the same nucleus. Figures 29 and 30 show the next stage with the accessory chromosomes in longitudinal and cross section respectively. The nuclear membrane has formed, the chromosomes are resolved into granules distributed on the nuclear reticulum and complete division of the cell body gives rise to two daughter cells, the secondary spermatocytes. Even at a very late stage of this process remains of the interzonal fibers, with the cell-plate, are conspicuous. In the late prophase of the secondary spermatocytes there arises from the preceding resting-stage a number of slender, twisted chromosomes closely resembling those which entered the resting stage and not infrequently the accessory chromosomes show a precocious, longitudinal split (Fig. 31). As the ordinary chromosomes are drawn into the equatorial plate prior to the second maturation division, their V-shape becomes evident. The apex of the V is the point of attachment of the spindle fibers, and the free, sinuous arms extend in various directions away from the spindle axis, giving a bushy appearance to the mitotic figure (Fig. 32).

On account of this arrangement of the ordinary chromosomes it is now extremely difficult to identify the accessory chromosomes. Also the fact that they are present in only half of the secondary spermatocytes lessens the chances of finding sections cut in a favorable plane for their identification. In spite of these disadvantages they can in some cases be clearly seen at the equator of the spindle where they lie near together and at right angles to the spindle axis. Sometimes indications of the longitudinal split can be detected (Fig. 33).

The arms of the V-shaped chromosomes shorten and thicken while they also become straight and densely staining. The plane of division passes through the apex of the V's and the rod-like arms move to the opposite poles. The accessory chromosomes divide along the line of the longitudinal split and their resultant halves pass to the opposite poles a little more slowly than the ordinary chromosomes. They are also distinguishable by their larger size. It is now apparent that one half of the spermatids will be "favored cells," containing two accessory chromosomes, while the other half will not be favored (Figs. 34–39). In polar views of the anaphase attempts were made to determine the chromosomal number but after the utmost care I can give only the probable number. In the majority of cases twenty-five chromosomes were counted (Fig. 37), and their straight, rod-like form makes it improbable that any of them were counted twice. Occasionally twenty-four, twenty-six or twenty-seven were counted, all of them appearing to be ordinary chromosomes. It seems now as if the reduced number must be at least twenty-five, instead of nineteen as given in my previous paper. In telophase, before the nuclear membrane forms, the ordinary chromosomes again become slightly sinuous in outline. The daughter cells must often move through a considerable arc in the process of separating, as sections are found showing two cells not yet completely seperated and at the same time showing approximately polar views of their respective chromosomes all in one plane. The accessory chromosomes appear thick and heavy at this stage (Figs. 40 and 41). Later the ordinary chromosomes are lost to view in the chromatin reticulum and give rise to the resting nuclei of the spermatids.

Transformation of the Spermatids.

One marked characteristic of the spermatids is that complete separation of sister cells is long deferred and in the early stages the cell-plate and interzonal fibers are conspicuous in the cytoplasmic neck connecting the two cells (Figs. 42 and 43). Near the cell periphery lies the centrosome from which an extracellular axial filament has grown out and this filament bears at the center and at the tip a transparent vesicle which stains deeply in iron-hæmatoxylin. The accessory chromosomes always lie near the distal pole of the nucleus with their outer ends turned toward the centrosome, so that here again we have the cell-polarity as beautifully shown as it is in the spireme stage. This ability to orient the cell brings to light the fact, already referred to, that sister spermatids must often undergo considerable rotation when they are drawing apart. In Figs. 44 and 45 the interzonal fibers have apparently disappeared but in all probability they give rise to the idiozome as claimed by Bösenberg ('05). At the center of the cytoplasmic neck connecting sister spermatids there is frequently a more or less evident enlargement which is of sufficiently general occurrence to deserve mention and in its center one finds the persistent mid-body (Zwischenkörper) even at a late stage (Figs. 46–51). This enlarged portion of the neck was called by Wagner the "connecting-body."

The nucleus now takes a form which bears a strong resemblance to the contraction phase of the growth period and were it not that it is of general occurrence in beautifully fixed material, it might be thought due to the harmful action of the fixing fluid. The chromatin reticulum contracts toward the distal pole of the nucleus into a mass which stains intensely, while the greater part of the nuclear cavity is left empty or is filled with nuclear sap. The accessory chromosomes lie in a distinct vesicle or at least in a clear space which gives them prominence and although they are closely pressed together the double nature of this nucleolus-like mass is easily demonstrated after long extraction in iron-alum (Figs. 46 and 47). As to what happens during this process of contraction I am wholly in the dark but later the nuclear cavity is fully occupied by a delicate reticulum upon which the chromatin granules are distributed in such a finely divided condition that they show very slight affinity for staining reagents (Fig. 48). The centrosome has divided into a proximal and distal portion and the proximal centrosome has moved some distance over the nucleus or has possibly entered into its interior. During its passage it gives rise to an intra-cellular filament which connects the proximal and distal centrosomes. The extra-cellular axial filament is now larger and its vesicles have increased noticeably in size. The accessory chromosomes are no longer inclosed in a vesicle but unite side by side into a single, elongated rod which leaves its former position at the distal pole and travels to the central pole in a line nearly or quite parallel with the cell-axis (Fig. 49). Following this stage the nucleus changes in outline, becoming somewhat pear-shaped and the proximal centrosome, which has become large and irregular in form, has passed over about one half of the length of the nucleus. The chromatin shows a tendency to collect at one side of the nucleus to form the chromatin plate. In half of the spermatids the fused or nearly fused accessory chromosomes occupy the center of the chromatin plate, extending from the central to the distal pole of the nucleus, or, in other words, from the anterior to the posterior end of the rapidly forming spermatozoön head (Figs. 50 and 51). The chromatin plate increases in size until all of the chromatin reticulum is involved; the whole nucleus becomes much longer than broad with the extremities slightly curved. The sister spermatids now separate completely, the rupture occurring on each side of the "connecting-body" when it is present. Figure 53 shows a somewhat later stage where the transformation is complete. The pear-shaped nucleus of the spermatid has been transformed into the crescent-shaped head of the spermatozoön and the chromatin is so compact that the whole head has a dark, grayish hue after long extraction in iron-alum. Even at this late stage the dimorphism of the spermatozoa is not concealed, for in half of them can be seen a slender, darkly stained band extending along the middle of the convex surface of the head, from the anterior to the posterior end, although it fades out often near the hinder extremity. This chromatic band represents the fused and somewhat modified accessory chromosomes whose distribution to but one half of the spermatozoa divides them into two distinct groups (Figs. 53 and 54).

In the mature spermatozoön the distal centrosome is no more in evidence, the axial filament has increased in length and its vesicles have disappeared. Whether or not the vesicles contribute their substance to the axial filament as it grows in length and whether or not the latter is supplied with a cytoplasmic investment, I am unable to say. The proximal centrosome forms a slight projection on the lower side of the head and probably corresponds to the end-knob of other forms. Wagner described it as a "little tooth" which lies at the point where the axial filament joins the chromatin plate and Bösenberg regards it as the middle piece, or rather as the "connecting piece," the former term not being applicable in the case of the spider spermatozoön. At the anterior end of the head is a transparent, apparently cylindrical body which in *Lycosa*, according to Bösenberg, is derived from the idiozome vesicle. Forming an axis in this apical body is a distinct fiber or filament which projects beyond the apical body and bears a deeply staining granule at its extremity. I cannot state positively the origin of the apical granule or of the filament which bears it but in some preparations, after long extraction, there is seen what appears to be a delicate filament passing from the end-knob, through the anterior portion of the head, and becoming continuous with the filament in the apical body. The distinctness of the filament within the head is exaggerated in the figures.

The spermatozoön now works itself free from the cell-body, the anterior end of the head protruding first. The escape is probably effected by the contractions of the head itself. Even after the posterior end of the head has entirely lost its connection with the cell-body, the spermatozoön is not yet ready to pass into the lumen of the testis (Fig. 55). First there occurs a very perceptible decrease in size through a closer and closer crowding of the chromatin granules which compose the head and through contraction of the nuclear membrane which incloses them. As the contraction progresses the staining capacity diminishes, in iron-hæmatoxylin preparations, and the spermatozoön head has a solid, grayish appearance. The accessory chromosomes, however, can still be recognized in the purplish band on the convex side of the head. In the second place, when reduction in size is at an end, the interesting process of rolling or coiling begins. The anterior and posterior ends of the head bend toward one another until they overlap to form a ring-like or disk-like body which well conceals the actual structure (Fig. 56). During the rolling up process Wagner believed that the tail coiled itself into a little, matted clump near the "tooth" (end-knob) and was therefore finally inclosed at the center of the ring when the rolling of the head was completed. Bösenberg, on the other hand, thinks that by careful focusing he can detect the tail wrapped around the outer circumference of the ring. My own observations lead me to agree with Wagner on this point for in partially coiled spermatozoa I have seen an extremely small, darkly stained mass which apparently depends from the end-knob and I have seen no evidence of a tail wrapped around the outer circumference. Since both Wagner's work and my own has been done chiefly on *Agalena* and Bösenberg's descriptions mainly refer to *Lycosa*, it is quite possible that both opinions are correct and that the method of disposing of the tail differs in the two genera. When all of the spermatozoa in a given cyst have almost or altogether completed the process of coiling, the cyst-wall ruptures and allows them to escape into the lumen of the testis and later into the sperm ducts. Even after they have been stored in the pedipalps they remain coiled but can be forced to uncoil if spread on a glass slide and heated to the boiling point.

If the spermatozoa in the proximal portion of the ducts be compared with those which have passed into the more distal portions, or with those in the pedipalps, there will be noticed a marked difference in their appearance. Instead of retaining the ring-like form, they become decidedly longer than broad and in fixed material the chromatin shows a tendency to shrink away from the outer wall leaving a clear area between it and the chromatin mass. Wagner described the final form of the spermatozoön as being rod-like and was criticized in this by Bösenberg who suggested that he was misled by seeing cross-sections of the disk-like sperm. This cannot possibly be the explanation for not only are all of the spermatozoa in the distal portion of the ducts and in the pedipalps in this elongated form but they are larger than when in the proximal portion of the ducts or in the lumen. This fact precludes the possibility of their being cross-sections (Fig. 59). Although they remain coiled they seem to lose the compact structure, which they assume before leaving the cysts, and regain their original size. This reëxpansion of the heads seem so improbable that I was only convinced of its truth after making numerous camera lucida drawings with utmost care. The difference in size and form can be seen at a glance by comparing Figs. 56 and 59 which were drawn with the same magnification. In all probability the spermatozoa first uncoil after they are passed through the slender ducts of the receptaculum seminis into the oviduct of the female spider.

When one considers that the tail is attached to the lower, anterior margin of the relatively heavy, crescent-shaped head, it is difficult to see how its lashing movements would propel the head in a straightforward direction. Bösenberg has made considerable study of the movements of spider spermatozoa and he holds that the chief propelling power is found in the twisting and bending motions of the head itself while the tail, by its lashing movements, may act as a steering-organ.

Sequence of Divisions.

In the maturation of the male germ cells it is generally conceded that two kinds of division occur — a reductional and an equational one — but in regard to their sequence there has been much difference of opinion. At a time when the majority favored the view that the equational division occurs first, Montgomery ('04, '05) maintained that the first division is reductional and he strongly emphasized the importance of determining the *origin* of the chromosomes in synapsis as their mere form in post-synapsis stages is often misleading. To-day perhaps the majority of workers can be said to favor Montgomery's view that the primary spermatocytic division results in a separation of maternal and paternal chromosomes which have conjugated in the synapsis stage. In my previous work I pointed out that *Agalena* offers especially favorable material for the investigation of this point and also that my results led to a full endorsement of Montgomery's interpretation. Further study has convinced me that in all probability that view is the correct one. Every one who has tried to follow the behavior of chromosomes during synapsis knows the difficulties in the way of reaching any certainty about the matter. What we do know is that, as a rule, before synapsis we find a certain number of chromosomes present and after synapsis we find but half that number. Many facts can be advanced in support of the theory of the conjugation of maternal and paternal chromosomes resulting in numerical reduction and if such a pairing of these nuclear elements does occur it may be brought about either by an end to end union of homologous chromosomes or by a union side by side. It is clearly of utmost importance to determine which method of union obtains before endeavoring to interpret what follows.

In *Agalena*, in the early prophase of the primary spermatocytes, the nucleus becomes contracted and its structure cannot be fully made out, but the chromatin seems to be in the form of a dense reticulum. At this time I believe the synapsis to occur both because there is no later stage in which there is any sign of its occurrence and also because the delicate spireme loops which issue from this stage appear to be in the reduced number. Strong evidence against the view that a side to side pairing of the chromosomes occurs is the fact that the spireme at first shows no trace of a split, then a barely perceptible one and finally quite a wide one running the whole length of each loop. The space intervening between two threads, therefore, must represent a longitudinal split and not the space between two threads about to unite side by side.

As evidence in support of the view that the chromosomes unite end to end, I would call attention to the noticeably denser structure of the chromatin at the bend of the loops which is quite marked in some stages, the chromomeres appearing to be massed together at this point. In iron-hæmatoxylin preparations the bend of the loops takes a deeper stain than the other portions do, while in safranin and gentian violet preparations the chromomeres at the bend of the loops take the safranin and the arms of the loops stain violet. Since condensed chromatin always does take the safranin, this differential staining may indicate merely a compact structure due to the mechanical bending of the loop but it may also indicate the junction of homologous chromosomes. In the light of the above facts, I consider it highly probable, therefore, that the split in the spireme represents a precocious longitudinal division which is more or less visible throughout the prophase of the first maturation division.

The succeeding steps are as follows: The longitudinally split loops become shorter and consequently thicker, drawing down toward the distal pole. The bend becomes acute, forming V-shaped chromosomes which split from apex to base along the line of the original longitudinal split and open out into double V's. At this point the chromosomes can be easily oriented for the split extends entirely through the free extremities of the arms of the V while the apices show the compact structure characteristic of the bend of the spireme loop. When they have taken up their final position at the equator of the spindle, one has no trouble in determining with certainty that the angles which correspond to the bend of the loop lie in the plane of division, while the angles corresponding to the free ends of the loop are directed to the opposite poles. Still further assurance, if needed, is gathered from the fact that sometimes a chromosome, which has not yet opened out, is drawn into the equator of the spindle and there always lies with its apex in the equatorial plane and its free ends directed toward the poles. Later this single V opens out into a double V preparatory to division. The first division then takes place through what corresponds to the apex of the original V-shaped chromosome and if this represents the point of union between the homologous elements, the first division must be reductional.

In the V-shaped chromosomes of the telophase, the space between the two arms corresponds to the split which first appears precociously in the spireme. When, in the succeeding division, the two arms are carried to opposite poles of the spindle, we plainly have a longitudinal or equational division. It is also of interest here that in this mitosis the rod-like accessory chromosomes divide equationally. To sum up, then, the first maturation division occurs at a point corresponding to the bend of the spireme loop and is a reductional one; the second maturation division passes along the line of the longitudinal split of the spireme loop and is an equational one.

Individuality of the Chromosomes.

Every living organism, whether plant or animal, single-celled or many-celled, is regarded as an individual notwithstanding the fact that each one is so lacking in stability that, in its metabolic processes, it has frequently been compared to a whirlpool into which and out of which new particles are constantly streaming. Again, in the ontogeny of a Metazoan, the cells of which it is composed and the cell-nuclei are supposed to be continuous from one cell generation to the next. When, however, we come down to one of the most important nuclear elements the chromosome, there has been much difference of opinion in regard to individuality, some claiming that when a given chromosome disintegrates and spreads out in a reticulum in the resting stage, the same chromosome does not reappear in the succeeding mitosis but that the chromosomes are formed anew each time. Ever since Rabl ('85) strongly supported by Boveri ('87, '88) and Van Beneden ('83) maintained that the chromosomes do not lose their individuality at the close of division but persist in the chromatic reticulum of the resting nucleus, scores of workers have brought forward evidence either for or against this theory. In a recent paper by Foot and Strobell ('07 (*b*)) we read as follows: If we mean by "Individuality of the Chromosomes" merely that we recognize certain characteristics of size and form in some of the chromatin units called chromosomes and that there is a frequent repetition of these forms during different stages of development, then we may claim that the chromosomes of *Anasa tristis* unqualifiedly support the theory of the "Individuality of the Chromosomes." But on the other hand, if by "Individuality of the Chromosomes" we claim their morphological continuity, that several or even only one of the chromosomes can be followed *uninterruptedly* from the spermatogonium to the spermatid, that even during the growth period the chromosome form is maintained, then we must say that in our preparations *Anasa tristis* supports in a very restricted sense, if at all, the theory of the " Individuality of the Chromosomes."

While all agree that the term individuality should not usually be taken in its narrowest sense in reference to chromosomes, the general fact that the same number of chromosomes issues from a reticulum as passes into it and that they have been seen to reappear in the same positions within the nucleus, added to strong evidence found in studies of fertilization of the egg of *Ascaris* and other forms, seem to me to clearly indicate genetic connection between the chromosomes in successive cell-generations. Even if the chromosomes do resolve themselves into their component granules which are distributed on a linin reticulum, it is not difficult to conceive of each one thus spreading out along definite lines, its ultimate branches temporarily anastomosing with those of adjacent ones, as a method of interchange of material or as a method of gaining nutriment for each granule which would be much less easily done in the dense, compact form. The probability is that the so-called resting stage is a stage in which physiological activity of the chromosomes is at its height. An *Amœba*, whether it be in an encysted form or whether it be spread out into a protoplasmic mass of extreme delicacy, with numerous pseudopodia, is still an individual *Amœba*.

In *Agalena*, the ordinary chromosomes offer no strong evidence in favor of chromosomal continuity although the loops of the spireme differ in length and in the prophase of the primary spermatocytes some of the V-shaped chromosomes differ slightly in size. One of these which opens at a much wider angle than the others recurs again and again, and is probably always present at this stage. When we turn to the accessory chromosomes, on the other hand, we find that they stand a more severe test than that outlined above and even comply with the demands of Foot and Strobell when they claim that, to meet the requirements for individuality, several or only one of the chromosomes should be followed uninterruptedly from the spermatogonium to the spermatid and that even during the growth period the chromosome form should be maintained. Not "only one" but *two* accessory chromosomes described in this paper have been followed without loss of identity from the spermatogonium through the growth stage, prophase, metaphase, telophase down to the spermatid and they more than meet the above requirements in that they have been traced to their final position in the head of the spermatozoön. The fact that they become granular and partially disintegrate for a short time in the growth period only shows that the granules, of which they are composed, separate from each other as in the other chromosomes but to a much less degree (Fig. 15). There is every probability that the other chromosomes have genetic continuity just as truly as the accessory chromosomes have it but at certain definite periods, possibly of great physiological activity, they take a form which temporarily obscures their individuality.

Degenerating Cells.

In my earliest studies upon *Agalena* I noticed many cases in which the mature spermatozoa seemed to be escaping from their respective cell-bodies and I then supposed that the latter could take no part in the formation of the germ-cells, but that they passed with them into the ducts and served as nutriment. Later, upon examination of some preparations made from another spider, *Pholcus phalangioides*, two kinds of degenerating cells were found in the lumen of the testis, one kind being supplied with brilliantly stained nuclei, while the other kind appeared granular and non-nucleated. These two kinds of cells were looked upon as early and late stages in the process of degeneration and the presence of chromatin precluded the possibility of their having originated from the cell-bodies discarded by the spermatozoa. The occurrence of spermatozoa wholly or partly free from their cell bodies was thus explained as a mechanical effect of sectioning with a microtome knife as it seemed likely that compact, resistant bodies like the sperm-cells might thus happen to be dislodged from the soft, protoplasmic mass in which they lie. Whence, then, came the great number of degenerating cells in the lumen of the testis and in the ducts? An answer to this question was sought in the unequal distribution of the accessory chromosomes. They were found to take no part in the first maturation division and, while their further history was extremely difficult to follow, I gathered, as I then believed, some evidence of their taking no part in the second maturation division, and therefore of their final distribution to but one fourth of the spermatozoa. McClung's theory that the accessory chromosomes might be sex-determinants was then held to be untenable, so far as its application to the spider was concerned, as it did not seem probable that one sex would be three times as numerous as the other. I then ventured to suggest a new theory viz.: that only the one fourth of the spermatozoa which contain the accessory chromosomes — the "favored" spermatozoa — become functional while the remaining three fourths degenerate after almost or altogether reaching maturity. There were *a priori* reasons for believing such to be the case since, if true, the parallelism between the spermatogenesis and the oōgenesis would be even more complete than hitherto supposed, three of every group of four daughter cells descended from a single spermatogonial cell being considered as homologues of the polar bodies which do not become functional. I also suggested, in view of the foreseen difficulties in the union of the sex-cells, that in the maturation of the egg the accessory chromosomes might be thrown off in the polar bodies and thus, at time of fertilization of the egg the normal chromosomal number would be restored. This second suggestion was overlooked by Boring ('07) in her criticism that if only the "favored" spermatozoa become functional, the egg must necessarily contain the accessory chromosomes also and that in the nucleus of the fertilized egg the chromosomal number would exceed the normal number by two. Berry ('06) in her paper on *Epeira* states that certainly in none of her preparations does she find any trace of degenerating spermatozoa, and other writers have expressed doubt of their existence. Now, while I still find an abundance of degenerating cells in the lumen of the testis and in the sperm ducts, my recent investigations have convinced me of the error of my former results in regard to the distribution of the accessory chromosomes. In the present paper I think it is clearly demonstrated beyond the shadow of a doubt that dimorphism of spermatozoa is the rule.

The above-mentioned facts have necessitated a careful reexamination of the whole subject of degenerating cells in the spider testis and after a brief review of the work of other writers, I shall give my present interpretation in the light of recent study upon this point.

Many of the earlier workers upon spider spermatogenesis have observed and described numerous, granular, protoplasmic bodies in the lumen of the testis, in the sperm ducts and pedipalps and also in the receptaculum seminis of the female spider. Most of them agree that these granules or granular masses have a nutritive function but they account for their origin in various ways. In *Tegeneria*, Bertkau ('77) believed that they arose from certain granular cells of the testis and sperm-ducts. Schimkewitch ('84) describes two kinds of cells in the testis of *Epeira*. Those at the posterior end, according to him, develop into spermatozoa. Those at the anterior end and also cells of the sperm ducts give rise to roundish or oval granules. In *Lycosa*, Birula ('94) found granular masses which were derived from the fragmentation of some of the follicle cells. On the other hand, Balbiani ('97) arrived at the conclusion that the spider testis is supplied with gland cells which pour out a secretion in the form of little granules. Wagner ('96*a*) maintained that the remains of the spindle fibers and Zwischenkörper fragment to form the "granules séminaux." Later appeared Bösenberg's paper ('05) in which I find statements which exactly accord with my earliest conclusions that the ripe sperm-cell works its way out of the cell-body and that the latter is left to degenerate.

"In der letzten Phasen der Umformung der Spermatiden in das Spermatozoön wies ich nach, dass der Kopf des Spermatozoöns mit dem Schwanz das Cytoplasma verlässt, welches dann in Form von grossen, runden Ballen im Follikel zurückbleibt. Diese Plasmakugeln degenerieren und zerfallen in kleine, runde Körnchen." Also, according to this author, the cells of the cyst-walls and their nuclei degenerate, the fragments of which pass into the lumen of the testis after the escape of the spermatozoa from the cysts. This whole mass of degenerating cells which completely envelop the rolled up spermatozoa in the ducts and pedipalps, Bösenberg regards as a possible source of nutriment for the sperm-cells until they reach the ova at the time of fertilization.

In my first study of *Pholcus phalangiodes*, already referred to, it will be remembered that two kinds of degenerating cells were found in the lumen of the testis, half containing nuclei and half not. I have now found that these bear no genetic relation to each other but arise in totally different ways. Those without nuclei are the cytoplasmic remains discarded by the spermatozoa and which accompany the latter when they pass into the lumen of the testis. Those containing distinct nuclei originate from degenerating spermatids, many cases occurring where nearly all or quite all of the spermatids in a cyst are in advanced stages of degeneration. The cell-body becomes enlarged and vacuolated and the chromatin forms homogeneous-looking masses irregular in outline or fragmented (Fig. 60). These cells greatly decrease in circumference, the chromatin mass becomes spherical, and the cell body becomes so transparent that it is easy to overlook it altogether. They vary considerably in size, even after they have passed into the lumen of the testis but they are readily distinguished from the ripe spermatozoa and the granular cytoplasmic bodies among which they lie (Fig. 61).

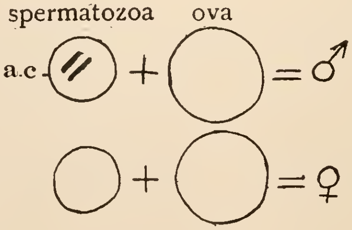
In *Agalena*, although in the breeding season the sperm ducts are fairly packed with nearly colorless cells or fragments of cells in which the ripe spermatozoa lie embedded, none of the former appear to contain chromatin, or, if they do, it is so finely distributed that it stains very faintly. Neither in this spider have I found cysts full of degenerating spermatids. After a careful study of the cysts containing nearly mature or mature spermatozoa, I am thoroughly convinced that Bösenberg is correct in stating that the ripe sperm-cells wriggle out of the cell body and furthermore he claims to have actually witnessed the process in his examination of living cells. I have, in fixed material, found the sperm-cells in all stages of the process and the phenomenon is of too common and too general occurrence to be accounted for by the tearing action of the microtome-knife in sectioning. After the spermatozoa have wriggled themselves free, they remain in the cyst for some time before the rupture of its walls and during this time, while they are contracting and rolling up into a disk-like form, described before, the cell-bodies from which they have escaped, also gradually contract, the circumference becomes greatly reduced and the density of the cytoplasm gives it a purplish hue in iron-hæmatoxylin (Fig. 58, *a–c*). When the cyst-wall ruptures, these granular masses pass out in company with the ripe spermatozoa and of course equal them in number. The cells of the cyst-wall also break up and their fragments pass into the lumen of the testis. Still another contribution to the mass of degenerating cells comes from the remains of the "connecting body" or cytoplasmic neck which for a time unites two sister spermatids and which contains the Zwischenkörper. In spite of all these sources of supply enumerated above, a difficulty still presents itself in the effort to explain all of the degenerating cells in the sperm duct. Even when we grant that they arise in three ways, viz.: from the cells of the cyst-walls, from the granular, cytoplasmic masses discarded by the spermatozoa and from the connecting-bodies and their contained mid-bodies, still the number appears to be too enormous to be wholly accounted for in these ways. No doubt there is considerable fragmentation but the size of the majority of the cytoplasmic masses is at least equal to the size of the contracted masses as they escape from the cyst (compare Figs. 56 and 59) so that fragmentation is not a satisfactory explanation, especially as the total mass of them far exceeds the total mass of the spermatozoa. It might be thought probable that the spermatozoa would pass through the ducts more rapidly than the degenerating cells and so leave a relatively large number of them behind, but it is difficult to see how this could be true since the spermatozoa are rolled up and are, in all probability, entirely inert. The contraction of the wall of the sperm duct would surely propel the degenerating cells as rapidly as the rolled up, temporarily inactive spermatozoa.

In the duct are found some cells of a type quite distinct from those already described and which could not have arisen in any of the ways mentioned. These are comparatively large, somewhat oval cells and closely resemble the rolled up spermatozoa in size and outline but differ from them in showing little or no affinity for nuclear stains. If it seemed probable that a spermatozoön would remain coiled during the process of degeneration, then we might suppose that these large, faintly stained cells represent degenerating spermatozoa and as they seem to be about as numerous as the deeply stained spermatozoa their presence might mean that only one half of the latter become functional. It seems more probable, however, that a spermatozoön would uncoil in the process of degeneration and it also seems likely that the chromatin in the head would retain its staining capacity for a long time. I am, therefore, at a loss to explain the presence of these large, oval cells.

SIGNIFICANCE OF THE ACCESSORY CHROMOSOMES.

Until comparatively recent times the problem of sex-determination has been approached chiefly from the outside and much experimental work has been done in the attempt to prove that external factors, such as nutrition, temperature, etc., do influence sex. Within the last few years, however, minute cytological research has directed attention to internal factors, namely, to the nuclei of the germ-cells themselves, and there is now good reason to believe that the problem can be put on a morphological basis. McClung's ('02*b*) brilliant idea that dimorphism of the spermatozoa caused by the presence or absence of the accessory chromosomes might have a direct bearing on the determination of sex has been strongly supported by Wilson ('05*a*, *b*, *c*, '06) and Stevens ('05*b*, '06*b*) and, more recently, by Boring ('07) in their work upon insects. These authors find cases in which the somatic cells of the male contain one less chromosome than the somatic cells of the female or, in cases where one half of the spermatozoa contain a very small chromosome represented in the other half by a large chromosome, the somatic cells of the male and female show corresponding differences.

In the spider *Agalena nævia* I have shown that dimorphism of the spermatozoa obtains, one half of them having two accessory chromosomes and one half of them lacking these elements. The comparative number of chromosomes in the somatic cells of the two sexes could not be determined, but a comparison of the developing germ-cells was made with reference to the presence or absence of the accessory chromosomes. In the spireme stage of the growth period of the primary spermatocytes, the two rod-like accessory chromosomes are more conspicuous than at any other time during the development of the sperm-cells (Fig. 13). On the other hand, a study of the primary oöcytes at a corresponding stage reveals the fact that in them no trace of the accessory chromosomes can be found (Fig. 57). It might be contended that during the much longer resting stage of the oöcytes, the accessory chromosomes dissolve into the reticulum as do the ordinary chromosomes but an argument against such an interpretation is the fact that the same lack is evident even in the extremely small ovaries of very young spiders captured in June — two months before the breeding season. At *all* stages of the growth period, in the youngest as well as in the oldest oöcytes, no accessory chromosomes can be found. Now, while this is not in itself conclusive evidence that all of the cells of the female spider lack the accessory chromosomes, it seems *probable* that such is the case in the light of the work upon insects and in view of the fact that one half of the spider spermatozoa lack these two elements. Since the primary oöcytes have no accessory chromosomes, in all probability the mature eggs lack them also. If, then, an egg be fertilized by a spermatozoön possessing two accessory chromosomes, a male would be produced but if an egg be fertilized by a spermatozoön which does not possess them, a female would be produced. This interpretation brings the spider into line with the insects in support of the view that the accessory chromosomes may be directly connected with sex-determination, the main difference between the insects and spiders being that in the former the female has the greater number of chromosomes while in the latter the male is the "favored" one.



COMPARISON OF RESULTS.

The earliest work on spider spermatogenesis which was done under sufficiently modern methods to concern us here is that of Wagner ('96*b*), but his complete paper, published in the Russian language, is not accessible to me. From a preliminary report in a German periodical ('96*a*) and from several short reviews, I judge that his work has been rather comprehensive, including the history of the germ-cells from the early spermatogonia to the mature spermatozoa. His studies were mainly concerned with *Agalena* and it is therefore with special interest that I compare my results with his. In the spermatogonia he states that division does not occur according to the ordinary method of karyokinesis, nor is it amitotic, so one is puzzled to know what method of division he did observe. He also makes the surprising statement that the nuclei of the spermatocytes are much smaller than the nuclei of the spermatogonia of the last generation. In the growth period and also in the primary spermatocytic division Wagner finds a peculiar nucleolus and while his description is far from accurate, I have no hesitancy in saying that under this term he describes the accessory chromosomes. This so-called "nucleolus" has a compact, elliptical form and is always peripheral in position, never lying inside the spireme threads. In the succeeding division it divides either in the plane of the equatorial plate or nearer one pole and in the latter case it is cast out into the cytoplasm (!). My results show that while the accessory chromosomes are usually in a peripheral position, they occupy a more central position in the growth period and are then surrounded by the spireme threads. As to the accessory chromosomes — they do not divide at all in the first division but are carried over bodily into one of the two daughter cells.

Early workers described the spermatozoön as of a disk-like, aberrant form showing no resemblance to the ordinary type. Wagner was the first to discover that this peculiar looking spermatozoön, with apparently no organ of locomotion, does in reality essentially agree in its development with the ordinary type and possesses head, tail and apical body. He also demonstrated clearly that the disk-like form is due to the fact that the ripe spermatozoön rolls itself up in such a way that it is difficult to recognize its resemblance to a typical spermatozoön. In the transformation of the spermatid, he incorrectly explained the growth of the axial filament, holding that it first appears in the cytoplasm and later makes connection with the nucleus and he was also mistaken in believing that a portion of the nucleus takes no part in the formation of the spermatozoön head and later disappears. Wagner's work, on the whole, added much to our understanding of the peculiar spider spermatozoön and made a foundation for the more detailed work of Bösenberg which is reviewed below.

Montgomery's work ('05) on *Lycosa* follows in some detail the history of the spermatocytes, and as a number of my results differ from his, it seems worth while to enumerate the main points in which we disagree. A careful perusal of his text and figures, and my own observations on several different genera including *Lycosa*, lead me to believe that he has misinterpreted some points and that a further study of *Lycosa* will bring about greater harmony in our results.

1. In *Lycosa*, he says: "There is no rest stage at any period of spermatocytic history."

In *Agalena*, well-marked rest stages occur in both of the spermatocytes and in the spermatids.

2. In *Lycosa* "where the ends of two conjugated chromosomes come together is frequently found a slight notch or break which is a connecting band of linin."

In *Agalena* the point of union is marked by a greater accumulation of chromomeres at the bend of the spireme loop.

3. In *Lycosa* "the split in the prophase (of the first division) does not extend through the distal ends of the generally V-shaped loops."

In *Agalena* the split extends throughout the length of the loop.

4. In *Lycosa* the longitudinal split of the prophase of the first maturation division becomes "in some of the chromosomes a little wider than during post-synapsis but this happens with only a minority of the chromosomes in any nucleus and it is not a definitive stage in the structural change of every chromosome for the reason of its relative infrequency. Most of the chromosomes are straight or bent rods."

In *Agalena*, the widening of the longitudinal split at this stage is of universal occurrence in the ordinary chromosomes and is of first importance as a foreshadowing of the opening out of the single V-shaped chromosomes to form double V's, which is the definitive form of every ordinary chromosome. Furthermore Montgomery's own figures of the telophase show many V-shaped chromosomes and indicate that in *Lycosa*, also, the chromosomes of the metaphase are double V's. On examination of my own sections of *Lycosa*, I find this to be true.

5. In *Lycosa* "there is no intermediate cell-plate formed after the reduction division but after all other divisions."

In *Agalena*, the intermediate cell-plate is always found at this stage and is often conspicuous.

6. In *Lycosa* "the two univalent heterochromosomes conjugate side to side though their ends directed toward the distal nuclear pole are in closer touch than their opposite ends, in contrast to the behavior of the other chromosomes."

In *Agalena* the heterochromosomes unite, apparently, into a single mass and the union usually begins at the ends directed toward the central pole.

7. In *Lycosa* "the mode of division of the bivalent heterochromosomes was not positively determined" but in its formation "there is some evidence that the heterochromosome may behave like the others during the maturation mitoses, namely, that it may undergo a reductional division in the first and an equational division in the second mitosis. And we can say positively that the whole bivalent heterochromosome does not pass undivided into one of the second spermatocytes." (!)

In *Agalena* and several other genera the heterochromosomes clearly pass undivided into the secondary spermatocytes and in *Agalena*, at least, they are equationally divided in the second maturation mitosis.

Bösenberg's beautiful work ('05) on the spermatogenesis of the Arachnida is based chiefly upon a study of *Lycosa* and his observations begin with the telophase of the second maturation division, his work being largely confined to a detailed study of the transformation of the spermatid into the mature spermatozoön. Taking Wagner's results upon *Agalena* as a starting point, he follows with utmost care the development of the spermatid nucleus, centrosome and idiozome. He, like Wagner, undoubtedly mistook the compact accessory chromosomes for a nucleolus which is conspicuous and often surrounded by a clear area in the spermatids. According to Bösenberg, this nuclear element disappears prior to the formation of the chromatin plate at one side of the spermatid nucleus. As a matter of fact, however, the accessory chromosomes (" nucleolus ") form an important part of the chromatin plate in half of the spermatids, first stretching across the side of the nucleus where the chromatin granules later accumulate. Bösenberg traced the subdivision of the centrosome into proximal and distal portions. The distal centrosome migrates to the cell-periphery and from it grows out the delicate extracellular axial filament. The proximal centrosome moves over or through a portion of the nucleus and later becomes comparatively large and pear-shaped. It is then regarded as the connecting-piece, or middle-piece, the latter term being thought inappropriate in the spider spermatozoön. The apical body is derived from the idiozome vesicle and contains a filament which bears a small granule. The latter is derived from the connecting piece. My observations, so far as they have gone, indicate a close agreement between the transformation of the spermatid of *Lycosa* and that of *Agalena*.

To Berry ('06) belongs the credit of first reporting dimorphism of the spider spermatozoa although in her brief paper on *Epeira* she was not able to bring forward much data in support of this view. In the telophase of the last spermatogonial division, one chromosome appears to have no mate and is therefore regarded as the odd chromosome which persists as a single, univalent element in the rest stages, becoming longitudinally split in the spireme. In the first maturation mitosis, the odd chromosome is carried to but one pole and while it was not identified in the second maturation mitosis it is thought to divide along the line of the original longitudinal split, the resultant halves being carried to the opposite poles of the spindle. This view is supported by the fact that apparently one half of the spermatids contain a single chromatin mass while the other half do not.

Knowing by experience the difficulty of accurately counting numerous rod-like chromosomes which often overlap one another and knowing also the possibility that all of the chromosomes may not necessarily lie in the plane of a given section and may therefore be counted twice, it seems to me unwise to place much reliance upon the finding of an apparently unmated chromosome in the telophase of the last spermatogonia. Again, the split and unsplit odd chromosome of the rest stage and of the growth period may be two univalent heterochromosomes or accessory chromosomes before and after conjugation, as in *Agalena*. In the primary spermatocytic division of *Epeira* the split, odd chromosome which is carried to but one pole is probably the two univalent accessory chromosomes and while Berry surmised that the halves of this single element are separated in the secondary spermatocytic division, it is possible that each half of the odd chromosome, or, as I believe, each univalent element of the two accessory chromosomes, splits lengthwise and is equally distributed to the opposite poles. While Berry finds a single chromatin mass in half of the spermatids, I find in *Agalena* two accessory chromosomes in half of the spermatids. These, however, eventually fuse into a single mass, so there is no real discrepancy here. Our main point of issue is with the *origin* of the accessory chromosomes. If it be granted that they originate from two spermatogonial chromosomes rather than from one, then it is possible to interpret all of Berry's figures in such a way that the odd chromosome of *Epeira* and the two accessory chromosomes of *Agalena* will be seen to be nearly identical in behavior and fate. The forms of the ordinary chromosomes of *Epeira* in the prophase and metaphase of the first maturation division are somewhat obscure in the figures but are described as V's, rings, rods and crosses. Now one who is familiar with the double V-shaped chromosomes in other forms can readily see how they might appear as represented, especially if they are overstained or closely packed together. Furthermore, my own sections of *Epeira* show plainly that the definitive form of the chromosomes in the equatorial plate of the first maturation division is that of a double V.

It may not be out of place to mention here the two chief errors in my own previous work ('05). In the first place I failed to find the division of the accessory chromosomes in the second maturation mitosis and in the second place I failed to find the tail of the spermatozoön. These points have been fully discussed in the body of this paper.

Although I am fully aware that it is easy to read one's own interpretation into the work of others, nevertheless I am confident that the spermatogenesis of at least three genera of spiders — *Agalena*, *Epeira* and *Lycosa* — will be found to agree in all essential points.

SUMMARY.

1. In the spermatogonia the nuclei are unusually large. The chromosomes are rod-like and are probably at least fifty-two in number. Two of them appear different from the others and are regarded as the accessory chromosomes.

2. In the primary spermatocytes, the ordinary chromosomes conjugate end to end in synapsis to form V-shaped chromosomes These open out along the line of the longitudinal split of the spireme to form double V's and divide reductionally. In the early rest stage the two accessory chromosomes take the form of two chromatin nucleoli which later unite into a single chromatin nucleolus. At the beginning of the growth period they again take the form of two rods which later conjugate side to side during a small fraction of the growth period. In mitosis they pass over bodily into but one of the two daughter cells.

3. In the second spermatocytic division the V-shaped chromosomes and also the two rod-like accessory chromosomes divide equationally. The reduced number of the ordinary chromosomes is probably at least twenty-five.

4. The spermatozoön has a well-developed axial filament derived from the distal centrosome. The proximal centrosome gives rise to the end-knob.

5. There is dimorphism of the spermatozoa, half of them containing two accessory chromosomes and half of them lacking these elements.

6. Since the accessory chromosomes are more conspicuous during the growth period of the primary spermatocytes than at any other time and in the primary oöcytes no trace of them can be found and since the dimorphism of the spermatozoa is due to

the presence or absence of these peculiar elements, it seems probable that an egg fertilized by a spermatozoön possessing the accessory chromosomes develops into a male while an egg fertilized by a spermatozoön which lacks the accessory chromosomes develops into a female. The results of my work upon the spider, therefore, furnish further evidence in support of McClung's theory of sex-determination.

7. Degenerating cells or cell fragments which envelop the ripe spermatozoa in the sperm-ducts come from at least four different sources. These are as follows: (*a*) Broken down walls of empty cysts, (*b*) cell bodies from which the ripe spermatozoa have escaped, (*c*) "connecting bodies" of sister spermatids, and their contained mid-bodies, (*d*) large, oval cells which resemble the rolled up spermatozoa, in size and outline.

UNIVERSITY OF PENNSYLVANIA,
May, 1908.

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EXPLANATION OF PLATES.

All figures were drawn with the aid of a camera lucida under Bausch and Lomb one twelfth oil immersion and Zeiss Comp. oc. 12. at table level and in the plates are reduced about one eighth.

All not otherwise specified are taken from *Agalena nævia*.

Abbreviations: *a.c*, accessory chromosome; *a*, mature spermatozoön; *b*, degenerating cytoplasmic body; *c*, degenerating spermatid; *d*, degenerating spermatozoön (?); *y*, yolk nucleus.

EXPLANATION OF PLATE I.

FIGS. 1, 2. Spermatogonium. Metaphase.

FIGS. 3, 4. Spermatogonium. Anaphase.

FIGS. 5–7. Primary spermatocytes. Rest stage.

FIGS. 8, 9. Primary spermatocytes. Contraction stage.

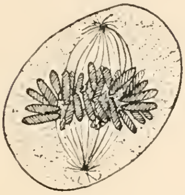
FIGS. 10–12. Primary spermatocytes of growth period. Spireme not split.

FIG. 13. Primary spermatocyte. Spireme thread longitudinally split.

FIG. 14. Primary spermatocyte. Cross-section of split spireme.

FIGS. 15–17. Primary spermatocytes. Split in spireme thread widens. The accessory chromosomes conjugate.

FIG. 18. Primary spermatocyte. Cross-section of split spireme and conjugated accessory chromosomes.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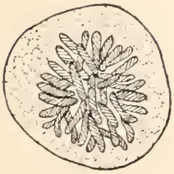
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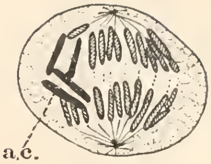

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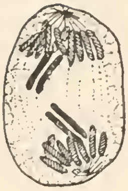
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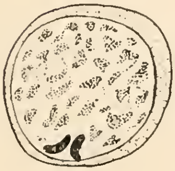
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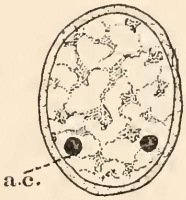

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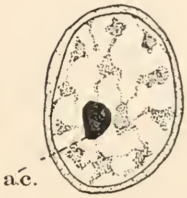

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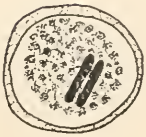
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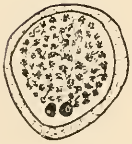
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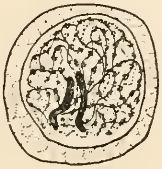
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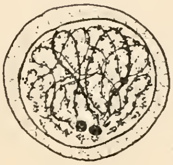
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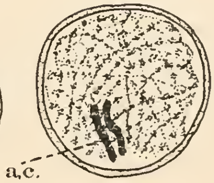

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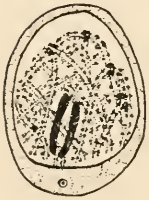
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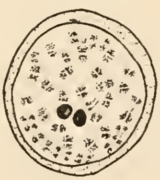
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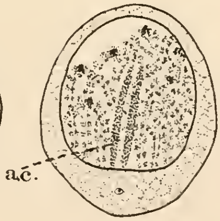

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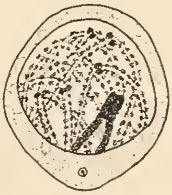
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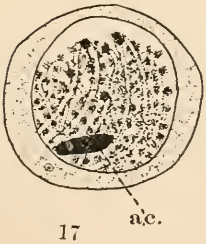

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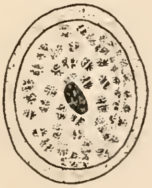
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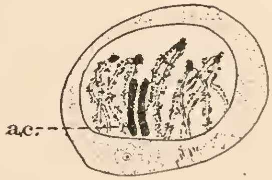

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EXPLANATION OF PLATE II.

FIGS. 19–21. Primary spermatocytes. The spireme loops contract. The accessory chromosomes separate again.

FIGS. 22, 23. Primary spermatocytes. Prophase. Single V-shaped chromosomes open out into double V's.

FIG. 24. Primary spermatocyte. Metaphase.

FIG. 25. Primary spermatocyte. Division of double V's to form single V's. Accessory chromosomes passing to one pole.

FIG. 26. Primary spermatocyte. Anaphase.

FIG. 27. Primary spermatocyte. Anaphase. Pole view, *a.c.* split.

FIG. 28. Primary spermatocyte. Telophase.

FIGS. 29, 30. Secondary spermatocytes. Rest stage.

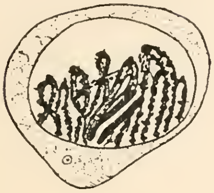
FIG. 31. Secondary spermatocyte. Prophase. Accessory chromosomes show precocious, longitudinal split.

FIGS. 32, 33. Secondary spermatocytes. Metaphase.

FIG. 34. Secondary spermatocyte. Division of V-shaped chromosomes into rod-shaped chromosomes.

FIGS. 35, 36. Secondary spermatocytes. Anaphase. Accessory chromosomes divide equationally.

FIG. 37. Secondary spermatocyte. Late anaphase, showing twenty-five rod-shaped chromosomes.



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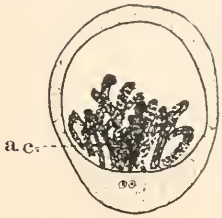

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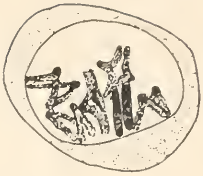

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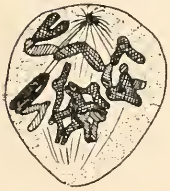

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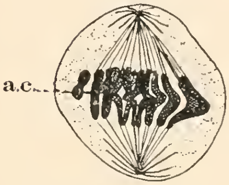

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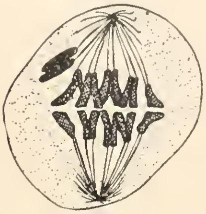

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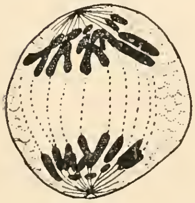

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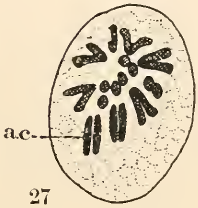

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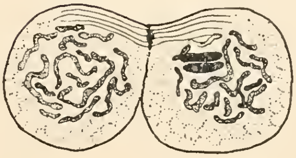

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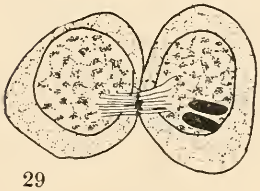

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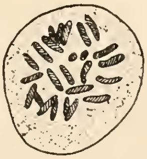

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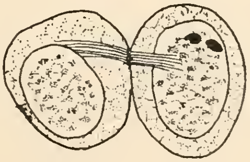

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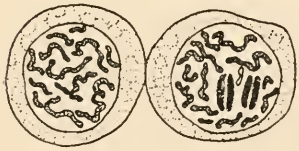

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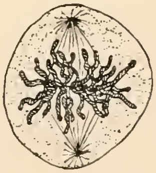

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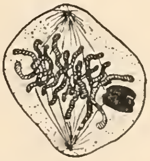

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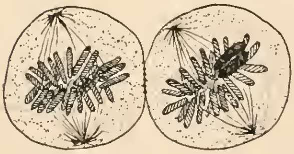

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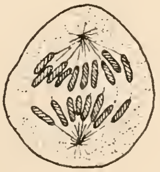

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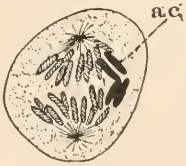

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EXPLANATION OF PLATE III.

FIGS. 38, 39. Secondary spermatocytes. Late anaphase.

FIGS. 40, 41. Secondary spermatocytes. Telophase. Accessory chromosomes large and conspicuous.

FIGS. 42, 43. Spermatids. Axial filament grows out from centrosome.

FIGS. 44, 45. Spermatids. Interzonal fibers disappear. The mid-body persists at center of "connecting-body."

FIGS. 46, 47. Spermatids. Contraction stage. Accessory chromosomes surrounded by clear area.

FIGS. 48, 49. Spermatids. Accessory chromosomes unite and pass toward central pole; proximal centrosome passes over or into nucleus. Axial filament lengthens.

FIGS. 50, 51. Spermatids. Nucleus elongated. Accessory chromosomes again show partial separation. Chromatin plate forms. Proximal centrosomes becomes larger.

FIG. 52. Spermatid. Cross-section of accessory chromosomes. Chromatin plate and proximal centrosome.

FIGS. 53, 54. Spermatozoa. Apical granule projects from apical body. Proximal centrosome becomes end-knob. Accessory chromosomes on convex side of head in Fig. 53.

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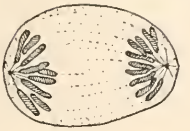

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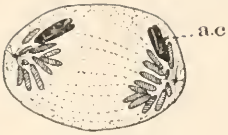

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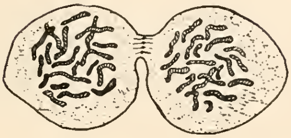

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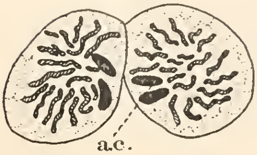

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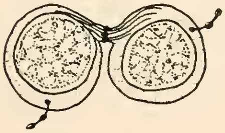

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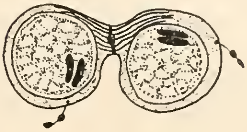

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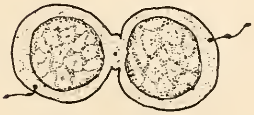

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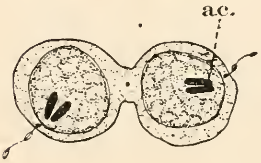

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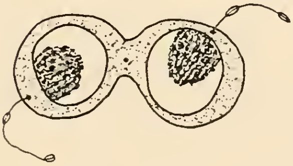

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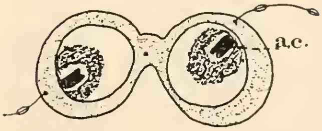

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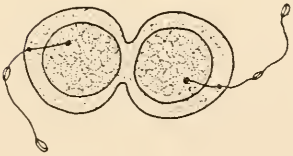

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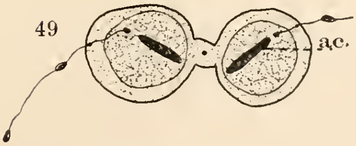

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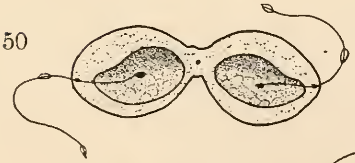

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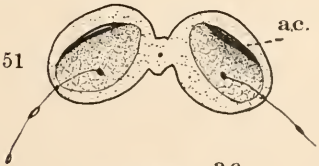

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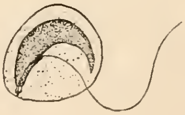

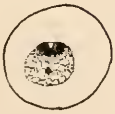

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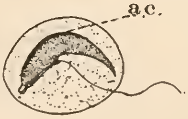


EXPLANATION OF PLATE IV.

FIG. 55. Spermatozoa escaped from cell bodies.

FIG. 56. Spermatozoa contracted and coiling up. Cell bodies contracting also.

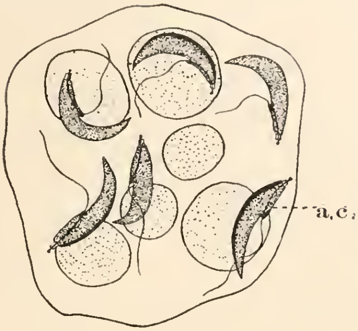
FIG. 57. Primary oöcyte, growth period.

FIG. 58. Cell bodies. Stages in degeneration, *a–e*.

FIG. 59. Portion of sperm-duct in breeding season.

FIG. 60. Cyst full of degenerating spermatids. (*Pholcus phalangioides.*)

FIG. 61. (*a*) Mature spermatozoa, (*b*) degenerating cell bodies and (*c*) degenerating spermatids found in lumen of testis of *Pholcus phalangioides*.



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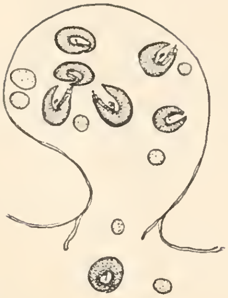

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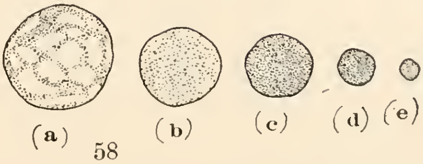

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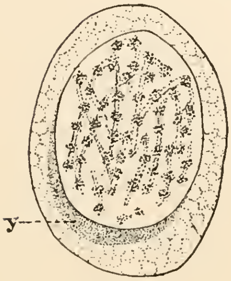

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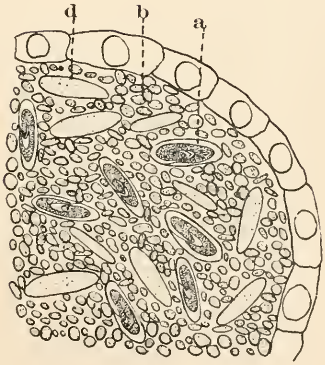

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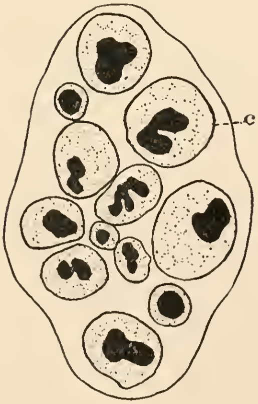

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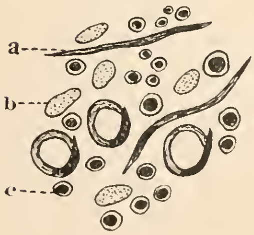

61

THE MOUND OF POGONOMYRMEX BADIUS LATRL. AND ITS RELATION TO THE BREEDING HABITS OF THE SPECIES.

C. H. TURNER.

Even casual observers, visiting the suburbs of Augusta, Ga., are sure to notice this ant ; not merely because of its large size, but on account of its conspicuous mounds, which are scattered, in open places, on all the vacant highlands. A somewhat dry, sandy soil seems best suited for its development ; for, although the mounds are numerous on the divide, both along the roadside and in open patches in the woods ; yet, they are practically absent from the low, damp flood-plain. The conspicuousness of each mound is not due to its height, for each is only a few inches high ; but to its large, barren surface. Some of the mounds are as much as five feet in diameter, and many have a major axis of three feet. The area of the mound seems to be a function of the size of the colony. I say size rather than age of the colony ; because an old, but weak, colony will often have a smaller mound than a much younger colony that has a large population. This condition of affairs is due to the habit of occasionally abandoning its old mound and excavating a new one. If the migration occurs when the colony is small, even though it is old, the area of the mound will be small. In small mounds the shape is often subcircular ; but in larger mounds the shape departs much from that of a circle (Figs. 1–8). This mound is composed partly of materials excavated in forming and enlarging the burrows and partly of small pebbles and other coarse materials collected from the surrounding surface of the ground. All sizes of workers participate in making the mound.

The number of openings into the nest varies from one to several. This is true not only of different nests, but of the same nest at different times. If an opening to a nest is closed with small particles, by some agency foreign to the nest, the ants that may be on the outside at the time act, for a time, as though they were confused; then they begin to remove the obstruction. In the meanwhile, the ants in the nest have been tunneling from within. Usually the ants from within are the first to penetrate the obstruction. Occasionally the ants on the outside are the first to make a breach. In one case observed by me, the ants on the outside took no part whatever in removing the obstruction until the ants in the nest had made an opening. When the nest opening is closed with anything which is not too large for several ants acting conjointly to remove, several will coöperate in removing it; otherwise a new opening is made. I have seen as many as five ants acting conjointly in removing an obstruction.

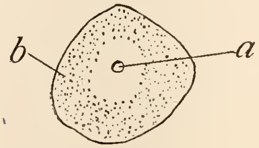

FIG. 1.

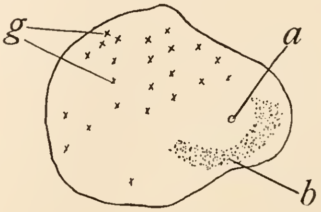

FIG. 2.

FIG. 1. Mound of *P. badius*, surface view. *a*, entrance; *b*, débris, mostly plant matter, from the nest. There were a few pebbles scattered over the mound. The nest contained workers of all sizes and several females. Greatest length, 17 in.; greatest width, 14 in.

FIG. 2. Mound of *P. badius*, surface view. *a*, entrance; *b*, débris, mostly plant matter, from the nest; *g*, stalks of green grass. The top is well covered with pebbles. Greatest length, 24 in.; greatest width, 20 in.

The statements in the preceeding paragraph were established by several experiments similar to the following: March 28, 1908, 5 P. M. — Numerous workers are on the outside of the nest. I fill the opening with sand and small pebbles. Immediatly the workers on the outside of the nest examine the obstruction and then rush about as though they are searching for an opening. 5:02 P. M. — Several workers try to force an opening through the obstruction. 5:10 P. M. — One of the workers confined to the nest succeeds in forcing a passage-way to the outside. Immediately three workers begin to remove the pebbles. Up until now, no particle has been removed from the outside of the nest. 5:15 P. M. — Another worker emerges from within. 5:20 P. M. — The opening has been cleared, and the ants are busy bringing from the nest the pebbles that the ants working from within the nest had carried inward from the obstruction.

Whether a nest contains several openings or only one, early in the morning and at night the openings are found to be closed. In the spring and early summer, I have often found all of the nest openings closed as late as 7:50 A. M. Careful observations, extending over a period of five months, demonstrated that the nest opening or openings are regularly closed, by the ants, at the close of day. The time required to complete the closing and the number of ants employed varies in different nests and in the same nest at different times; but the method in all cases is so similar that the following description of the behavior of the ants on one occasion will serve as a type of all.

It was eighteen minutes to seven, July 20, 1908, and darkness was fast approaching. Four workers were busy covering the nest opening with plant débris, sand and pebbles. Part of the time they would stand balanced upon the two posterior pairs of legs, with their heads turned away from the nest, and kick the sand backwards with their fore feet; and part of the time they would carry coarser materials and place them upon the nest. By twenty-three minutes after seven, the opening was almost closed and the ants passed inside. In a few minutes, two workers returned to the outside and continued the closing of the opening. Soon they were joined by a third worker. By forty minutes after seven the opening was closed; then the three workers wedged themselves inward through the closed entrance. Soon a worker returned to the outside and added more material to the pile over the nest opening. Then it forced its way into the nest. This behavior was repeated several times. The nest was finally closed from within. By eight o'clock everything was quiet.

On one occasion, I noticed this ant make use of this hole-closing instinct to rid itself of the encroachments of another ant. A species of *Atta* had constructed a burrow within the confines of a mound of a colony of *Pogonomyrmex badius*. When I first discovered these conditions, a single worker of the latter species was busy covering the opening of the burrow of *Atta* with sand. The sand was driven into the opening by the ant standing on its four hind legs and kicking the sand backward with its fore feet.

The larger particles were carried in the mouth and deposited therein. Every few moments the ant would enter the burrow and compact the sand with its head. Frequently it would leave the hole and wander about for a few moments. It always soon returned to the covering. After the hole had been filled to the level of the ground, the ant continued to pile on sand. When I left, at 10:03 A. M., the ant was still piling on sand. At no time was it assisted by any other ant, although numerous ants passed by it.

With few exceptions, on each mound there is one or more trash piles. In over two hundred mounds examined I noticed only one exception (Fig. 3). On this trash pile is dumped débris from the nest, supplemented by plant material obtained in the surrounding territory. The major portion of the pile is composed of plant matter, the remainder of remnants of insects. In some cases the trash-pile surrounds the nest-opening (Figs. 1, 5, 7, 8); but in most cases it does not seem to bear any definite relation to that opening (Figs. 2, 4, 6).

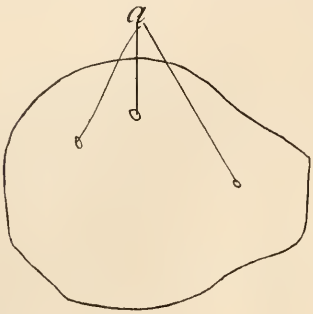

Fig. 3.

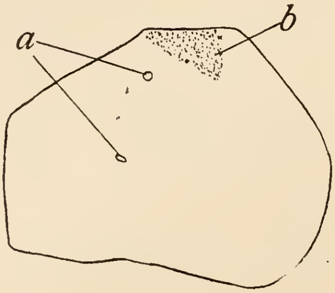

Fig. 4.

Fig. 3. Mound of *P. badius*. *a*, entrances. The mound is covered with pebbles, and dead stalks of grass and weeds are scattered over it. Greatest length, 32 in.; greatest width, 25 in.; greatest height, 5 in.

Fig. 4. Mound of *P. badius*, surface view. *a*, entrances; *b*, débris, mostly plant matter, from the nest. The mound is covered with pebbles. On the outer edge there are a few stalks of grass. At one time this mound had four openings. Greatest length, 32 in.; greatest width, 27 in.

It seems self-evident that a mound as conspicuous as this must play some important rôle in either the present or the past behavior of the species. It is possible that it serves more than one purpose; however, several months careful study has convinced me that one of the most important functions of this mound is to serve as a device to promote sexual union. The following description of the behavior of this species, during the process of mating, will make this point clear.

Mating takes place, at intervals, during the warm weather. How often this occurs and whether the intervals are regular or irregular I am not prepared to say. I observed the inmates of one set of nests mate on June 8 and again on June 16, and, on several other occasions, I saw what looked like signs of recent mating. Practically all the nests of the same locality mate at the same time. On one mating occasion, out of eleven nests occurring within a radius of a few yards, the inmates of eight were going through the mating behavior. The three colonies that were not mating were small and weak.

During the daylight hours, a few workers usually promenade on top of the mound; just before a mating is to occur, this number is increased many fold. To a casual observer, it looks as though the majority of the inmates of the nest were out for a sun bath. All move about in a nervous manner. A few males and a few unfertilized (winged) females repeatedly emerge from the nest, move about for a few moments, and then retire. After this has continued for a time, the males fly away and the females promenade among the restless workers. When the males arrive at another mound, they alight and move about, in a jerky, nervous manner, among the restless workers and females on the mound. If, in its zigzag rushing, the male comes in contact with one or more workers, these neuters attack it at once. Sometimes a male attempts to enter the nest. It is at once opposed by the workers. I have never seen a male succeed in entering the nest; but, I think they do sometimes; for, on more than one occasion, I have seen workers dragging males out of the nest. When a male comes in contact with a winged female, he mounts her back and attaches his reproductive organs to the tip of her abdomen. She either remains quiet, or else gently nibbles the abdomen of the male with her jaws. After a few minutes of intimate contact, the ants separate; each to repeat the act with some other ant. This coition always occurs either on the ground or on a blade of grass or a low weed; never in the air.

That the same female accepts the attentions of several males and that the same male serves several females I have established by experiments conducted as follows: A number of males, captured as soon as they alighted on the mound, were confined, by means of cotton plugs, in a small test-tube. In a second test-tube, was placed a single female. Into this second tube a single male was introduced. As soon as the pair had separated after coition, that male was removed and a fresh male introduced. This was repeated until the female refused to mate. A dozen experiments of this sort were conducted. The greatest number of males accepted by the same female was four; the least number was two. After the last successful coition, the introduced male always tried to copulate, but in vain. On the mound, on several different occasions, I have seen the same female copulate with two different males.

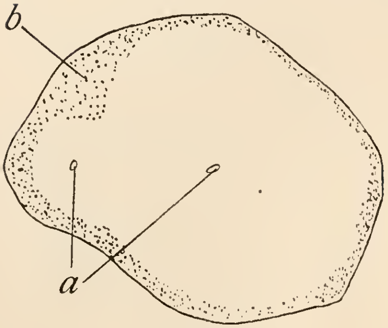

FIG. 5.

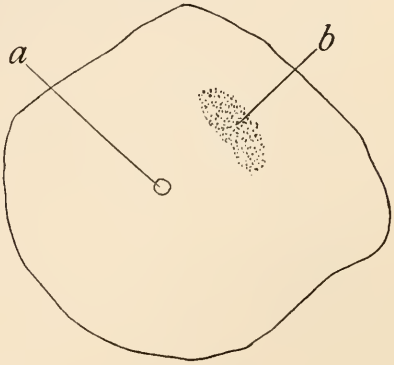

FIG. 6.

FIG. 5. Mound of *P. badius*, surface view. *a*, entrances; *b*, débris, mostly plant matter, from the nest. There is a depression around the main opening. Greatest length, 38 in.; greatest width, 31 in.

FIG. 6. Mound of *P. badius*, surface view. *a*, entrance; *b*, débris, mostly plant matter, from the nest. Scattered over the top of the nest, are numerous pebbles and bits of coal. Near the opening there is a depression which slopes towards the opening. Near the edge there are a few stalks of grass. Greatest length, 39 in.; greatest width, 34 in.

In another series of a dozen experiments, several females were removed from the top of the mound and confined in a cotton-stoppered test-tube. In another tube was confined a male that had just arrived at the mound. One of the females was introduced into the tube with the male. They copulated immediately. As soon as coition was over, the female was removed and a fresh female introduced. This was repeated until the male would no longer mate. The greatest number of females served by the same male was five; the least, four. After the last successful coition, when a fresh female was added, the male would attempt to copulate, but in vain.

These experiments show, conclusively, that the same female may mate with several different males and that the same male may serve several females. As to the number of services that either may give or accept, these experiments do not give a final answer; for there was no way of being sure that the female captured on the nest had not previously been served; nor could I be absolutely certain that the male had not visited some other mound and copulated before arriving at the nest.

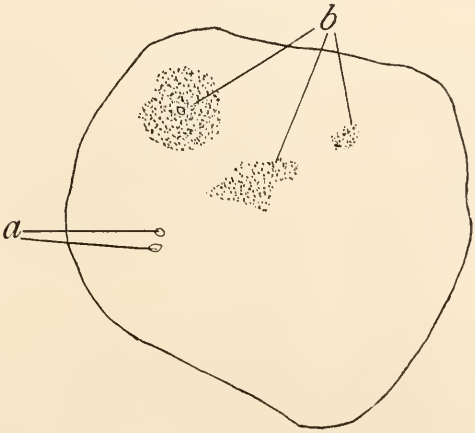

FIG. 7. Mound of *P. badius*, surface view. *a*, entrances; *b*, débris, mostly plant matter, from the nest. The top is covered with pebbles. On the edge there are a few stalks of grass. Greatest length, 40 in.; greatest width, 39 in.

From the above description, it seems evident that the mound of this species is a device for promoting cross-fertilization. The large, conspicuous, expanse of barren land in the midst of vegetation serves as a stimulus to attract the flying male. Whether this is to be regarded as a tropism or an ordinary reflex is more than the experiments answer. That it is an instinctive response is self-evident.

There is a wide-spread belief that the male ant always dies within a few hours after copulation. The following two experiments show that, in this species, the male may live for several days after mating.

June 8, 1908. — Five males, captured just after copulation, are confined to a Janet nest. June 10. — All are alive. June 11.— Two are dead and three living. June 12. — Three are dead and two living. June 16. — Four are dead and one is dying.

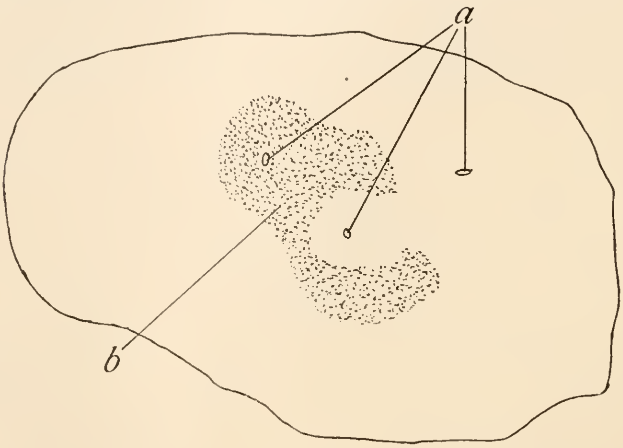

FIG. 8. Mound of *P. badius*, surface view. *a*, entrances ; *b*, débris, mostly plant matter, from the nest. The surface is covered with pebbles and bits of coal. Near the edge a few grass stalks are growing, and, scattered over the surface, a few stalks of dead grass are standing. Greatest length, 60 in.; greatest width, 42 in.

June 16, 1908. — Fifteen males, captured just after copulation, are confined in a Janet nest. June 17. — All are alive. June 18. — All are alive. June 19. — One is dead, one is dying, and thirteen are alive. This series was interrupted by a forced absence from the laboratory.

In each of the above cases no male died until at least two days had elapsed after copulation, and, in each case, some of the males lived for several days.

Females captured immediately after copulation and confined in a Janet nest, often did not shed their wings until at least two days had elapsed. This is not in harmony with the belief that all female ants shed their wings on the evening of the day on which copulation occurs.

Conclusions.

1. At the mating time, the females of *Pogonomyrmex badius* and the numerous workers roam about on their home mound; but the males fly away.

2. The broad, barren mound, situated in the midst of vegetation, arrests the males in their flight and thus promotes cross-fertilization.

3. Mating occurs on the ground or on a blade of grass or on a small, low weed; but never on the wing. This usually happens on the mound; but, sometimes, it occurs in the adjacent grass.

4. The same female may be impregnated by several males, and the same male may serve several females.

5. The male does not die until several days after mating.

6. The nest openings are closed by the ants at the close of every day.

Summer High School,
St. Louis, Mo.,
April 9, 1909.

CONTACT ORGANS IN THE KILLIFISHES OF WOODS HOLE.[1]

H. H. NEWMAN.

In a former paper[2] the writer had occasion to describe certain interesting structures occurring as a secondary sexual character in the spawning males of four species of Pœciliidæ occurring in the waters about Woods Hole, Mass. These structures were designated "contact organs," the writer venturing to use a new term for the reason that no very similar structure had been previously described. Contact organs seem to have a somewhat similar function to that of the pearl organs of other authors, but in structure they are utterly different.

Although these little organs have been observed on only four species, it seems highly probable that they may prove to be characteristic of the Pœciliidæ, and hence of some systematic importance.

Occurrence.

Contact organs are found on all spawning males of the following four species: *Fundulus heteroclitus*, *F. majalis*, *F. diaphanus* and *Cyprinodon variegatus*.

They occur regularly in the following regions:

1. On the rays of the dorsal and anal fins.
2. On the ventral fins in *Fundulus heteroclitus* and *F. diaphanus*.
3. On the margins of the scales of the sides, cheeks, snout and top of head. In *F. majalis* they also cover the back from the head to the dorsal fin.

These are the regions in the males that come into most intimate contact with the female during the spawning act proper or during the preliminary courtship. Differences as to the details of the distribution of these organs among the species are closely correlated with differences in the spawning attitude.

In *Fundulus heteroclitus* the spawning takes place in pairs, a female being clasped by only one male at a time. The two bodies are closely pressed together side to side, the tails turned considerably to one side and the heads to the other. The male clasps the female securely about the body back of the dorsal and anal fins, using as claspers his large, strong dorsal and anal. For additional security the small ventral fins on the sides of contact are locked. On the body the points of closest contact are the region of the sides between dorsal and anal fins and the side of the head, which is bent sharply against that of the female. The distribution of the contact organs is in detail just what might be expected on the supposition that the name given them is apt. The organs are largest and most numerous on the proximal parts of the dorsal and anal fin rays and on a rather narrow zone of the body immediately between these two fins. They are fairly

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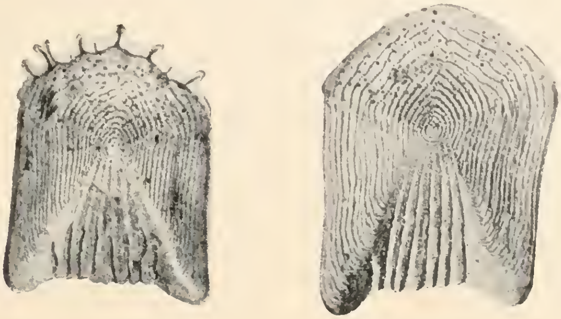
[1] Contributions from the Zoölogical Laboratory, University of Texas, No. 97.

[2] H. H. Newman, Biological Bulletin, Vol. XII., No. 5.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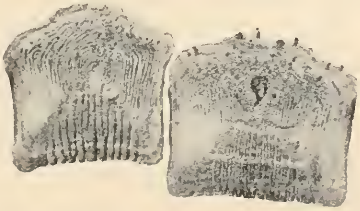
Photographs showing scales with and without contact organs, × 12.

FIG. 1. Scale from the side of a large male *F. majalis.*

FIG. 2. Scale from a corresponding part of the body of a large female *F. majalis.*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Photographs showing scales with and without contact organs, × 12.

FIG. 3. Scale from the lateral line region between the dorsal and anal fins of a spawning male *Cyprinodon variegatus.*

FIG. 4. Scale from a similar location of a spawning female *Cyprinodon variegatus.*

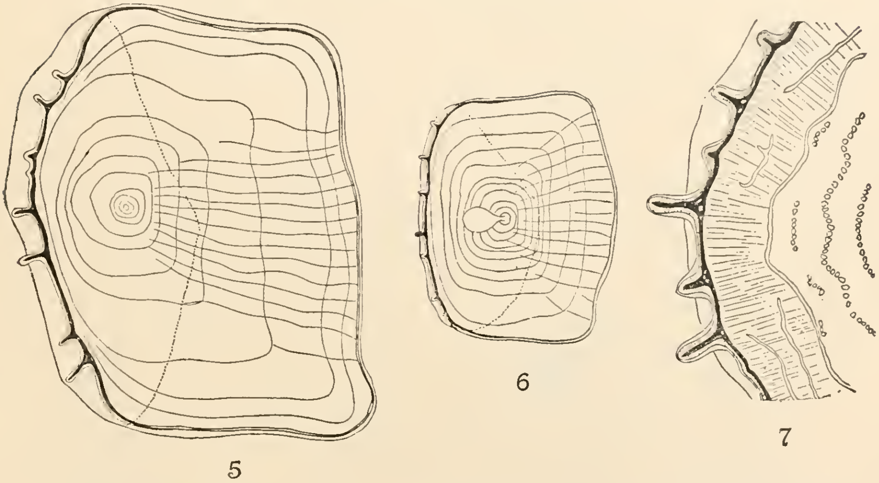
large and numerous on the sides of the head and on the ventral fins, and comparatively small and scattering on the sides anterior and posterior to the point of greatest pressure.

The distribution of the contact organs in *F. diaphanus* is strikingly like that just described for *F. heteroclitus*. The spawning attitude has not been observed, but, from analogy, one might be fairly certain that it closely resembles that of the latter.

F. majalis seldom spawns in captivity and when the former paper was written the writer had never observed a case of spawning in this species. During the following summer, however, several instances of this phenomenon came under observation. *F. majalis* does not seem to spawn in pairs, but two or more males coöperate with a single female. On one instance as many as five males were seen piled in a struggling heap over one female. The whole mass was in strong vibration and the surrounding water was clouded with milt. Although the exact details of the spawning attitude could not be made out on any occasion, it is certain that the points of contact are much less restricted than in *F. heteroclitus*. The nearest males clasp convenient parts of the female and the outer males seemed to clasp the inner ones or to crowd their bodies into the mass backforemost. The distribution of contact organs accords with this rather promiscuous manner of spawning. They are largest and most numerous on dorsal and anal fins and on a zone of the body between these fins. There are none on the ventral fins as they could not be locked in spawning. On the dorsal surface of body and head, on cheeks and snout, they vary in size and abundance, but in many specimens the ventral surface of the body and the ventral and pectoral fins are the only parts not provided with them.

Cyprinodon variegatus, a small species with decidedly compressed body, spawns in pairs in much the same fashion as *F. heteroclitus*, but the short body and very flat sides give a greater area of contact between male and female. Corresponding with this greater area of contact the contact organs are more generally distributed over the body than in any of the other species. On the very high dorsal fin, however, they are restricted to the proximal portion of the rays, for only that portion can touch the sharp-edged back of the female. They occur plentifully on the smaller anal and ventral fins.

In all species the contact organs occur on the top of the head and upon the snout, places where there is little or no direct contact in spawning proper. These regions, however, are used constantly as contact surfaces during courtship and rivalry. During courtship the male swims beneath the female and seems to guide her about from place to place by gently "butting" her with the top or sides of the head. Rivalry in at least two of the species, *F. heteroclitus* and *Cyprinodon variegatus*, is very intense. Two males rush at one another head on and strike one another savagely with cheeks, jaws and snouts. Such contests may be kept up at intervals for days. A number of specimens were examined after they had been engaged in combat for some time to determine the effect of fighting upon the contact organs. In all cases the latter were found to be much worn, many showing the supporting spike stripped of dermis and epidermis. Apart from



Camera drawings of typical scales from the region between the dorsal and anal fins of spawning males.

FIG. 5. *Fundulus heteroclitus*, × 16.

FIG. 6. *F. diaphanus*, × 16.

FIG. 7. Enlarged detail drawing of a portion of the exposed margin of a scale taken from the side of a male *F. majalis*. The black region represents the horny margin of the growing region of the scale that is prolonged into spikes that support the contact organs. The stippled area represents the dermis. The clear outside area represents the epidermis. The striated portion in the non-calcified portion of the scale, × 30.

this no damage seems to have been suffered. No doubt the violent contact of these organs is somewhat painful to both parties in the struggle, for, judging by their nerve supply, they must be very sensitive.

For diagrams showing the average distribution of contact organs in spawning males of the four species see Text Plate II. of the paper previously referred to.

Arrangement and Position of Contact Organs on Scales and Fins.

On the Scales. — At the height of the spawning season they occupy the entire free edge of the scales on which they occur, standing out like curved fingers at an angle of about thirty degrees from the flat surface of the scale. The scales of the body proper have only an arc of their circumference free and hence can produce contact organs only on this free surface (see Figs. 1, 3, 5 and 6). On the head and cheeks, however, many of the scales are plate-like with the whole circumference free. In the latter case contact organs occur around the whole margin. Fig. 8 shows in detail the distribution of the organs on the head of typical male of *F. majalis*, in which the scales show varying amounts of

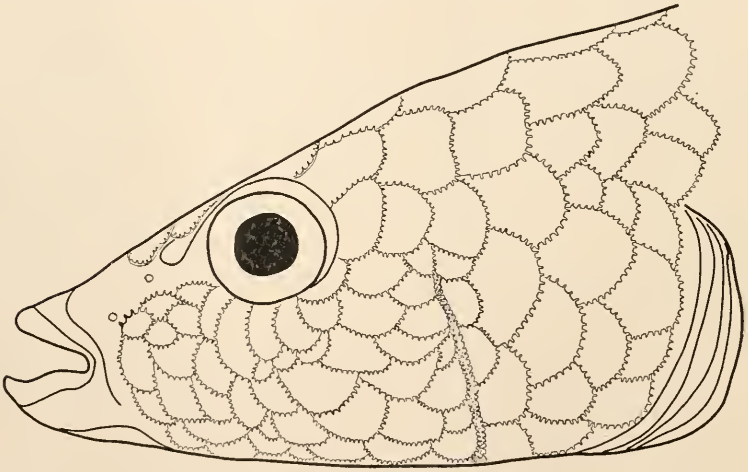


Fig. 8. Camera drawing (×4) of the head of a male *Fundulus majalis* with complete equipment of contact organs. Notice that the latter occur at fairly regular intervals along all free margins of the scales.

free margin. The only scales with entirely free margin shown here are certain small ones on the snout. On top of the head, however, there are several large plates with the entire margin free.

On the Fins. — Fig. 9 is a camera drawing showing the distribution of the organs on the anal fin of a large male *F. majalis.* It will be noted that they are largest and most numerous on the ends of the rays of the small ventral lobe, the part most closely pressed against the body of the female in spawning. They occur as branches from the bony fin rays, usually one or two to the joint, and frequently form clusters at the distal end of the rays where branching is occurring. Fig. 10 shows a condition of this sort on one ray of the fin shown in the previous figure, where it was impossible to show all of the elements in the terminal cluster. The portion represented in solid black is the bony portion. It will be noted also that the majority of the contact organs are slightly hooked toward the base of the fin. This position should

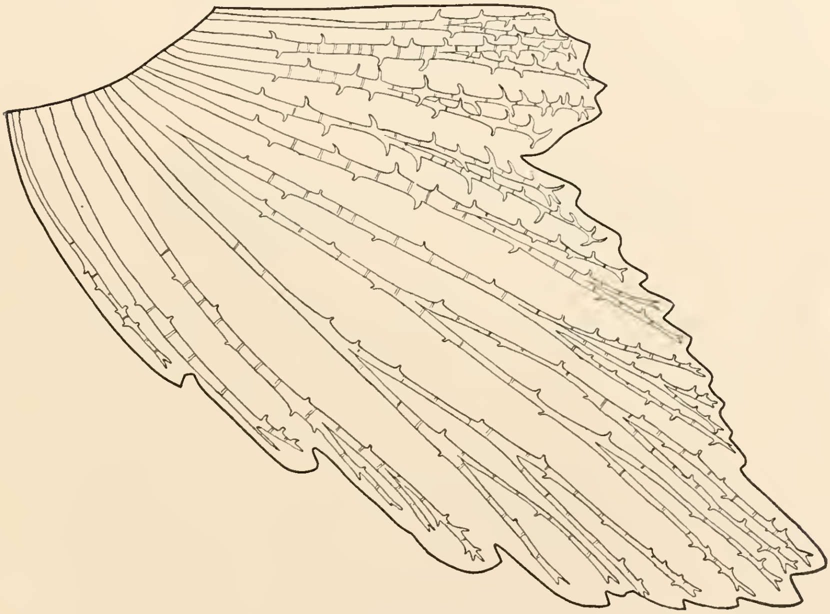


FIG. 9. Camera drawing (× 4) of the ventral fin of a very large male *F. majalis.* The preparation was cleared in strong KOH solution.

be effective in enabling the male to hold the female more securely. In some specimens of *F. majalis* the organs are restricted to the small posterior lobe. From the few observations made thus far on the spawning attitude of this species it would seem that in many cases the anal fin can play only a minor part in clasping. Hence it may be that there is at present a tendency to reduce the number of organs on this fin. The writer takes occasion to point out this weak spot in the evidence in favor of the general conclusion that the distribution of contact organs is intimately correlated with the spawning attitude. The possible explanation may lie in the fact that only a very few observations have been made of spawning in this species, and the attitudes then seen may depart somewhat from those assumed in the open. All observations agree, however, as to the promiscuity of the spawning. In both *F. heteroclitus* and *Cyprinodon variegatus*, in which all of the details of the spawning attitude have been accurately determined, the correlation between spawning attitude and distribution of the contact organs holds without exception. The relationship of the organs to the fin rays is the same, with minor differences, in all species studied.

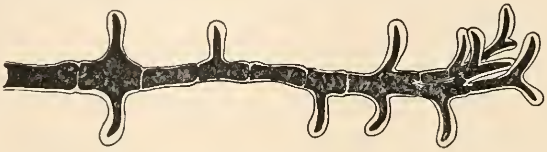


FIG. 10. Camera drawing (× 12) of the distal portion of a single ray from the posterior lobe of the anal fin of a spawning male *F. majalis*. The solid black represents the osseous structure of fin ray and contact organs, the single line the boundary of the epidermal portions.

Structure. — Attempts to section the contact organs were disappointing. After complete decalcification there still remained in the central supporting spikes crystals of some hard substance, probably guanin, that resisted the knife edge and tore through the section. Fragments of tissue from such sections revealed the fact that the epidermal covering was thin and of the usual type and that the dermal layer was much thicker and was composed of rather loosely arranged connective tissue, blood vessels and a few nerve fibers. A substitute method was found, however, that served to reveal the essential histological structure almost as well as the usual method of sectioning and staining. Scales or fins were soaked for a day or more in rather strong KOH solution. This treatment renders all tissues except the osseous parts quite transparent, so that, by carefully manipulating the illumination, practically all details were revealed under low powers of the microscope.

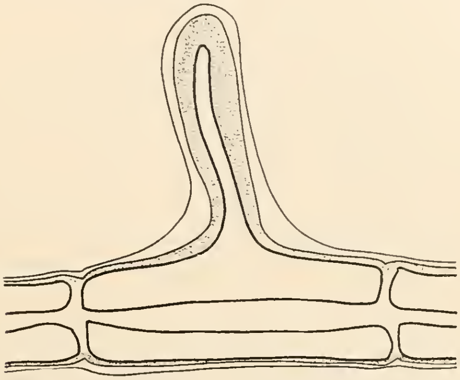


FIG. 11. Camera drawing (× 60) of a single contact organ on a ray of the anal fin of a male *F. majalis*, cleared in KOH solution. The unshaded central portion represents the osseous portion of fin ray and contact organ, showing the continuity of the two. The stippled region is the dermis overlying the osseous portions. The unshaded marginal portion represents the epidermis.

On the scales (see Fig. 7) the spike-like core is continuous with the newest layer of osseous material, while on the fins (see Fig. 11) the core is of a piece with the outer layer of the fin rays. In other respects the organs are alike in both locations. Surrounding the core which is dense and hard, although somewhat elastic, is a thick layer of dermis, more abundant near the tip of the organ. The comparatively thin epidermis seemed to be stretched over the tip of the organ and was frequently broken. Near the base, however, it was as thick or thicker than the dermis of that region. The difference between these structures and pearl organs may be seen at once in the comparatively secondary importance of the epidermis and in the core of dermis with its osseous support. The pearl organ is merely an epidermal callosity.

ORIGIN, DEVELOPMENT AND DISAPPEARANCE.

In order to determine how early in the season the contact organs begin to make their appearance, a number of specimens of the two species most accessible, *F. heteroclitus* and *F. majalis*, were very kindly collected for me by Mr. G. M. Gray, of the Woods Hole supply department. The results of the examination of these specimens are shown in the following tables:

TABLE I.

(*F. heteroclitus* males, collected April 27, 1907.)

No. of Specimens.	Distribution of Contact Organs.
5	A few on the posterior lobe of the anal fin.
3	Fairly numerous on the tips of nearly all of the rays of the anal fin.
1	Plentiful on the rays of the posterior lobe of the anal fin.
1	Scattering distribution over nearly the whole area of the anal fin.
1	Numerous on all of the rays of the posterior two thirds of the anal fin.
8	None.

According to these data the earliest appearance of contact organs in this species is on the anal fin, especially on the posterior lobe of the latter. They appear first upon the distal ends of the rays and from there extend gradually toward the base. One is tempted to suggest a correlation between the early appearance and the functional importance of these organs. There seems to be no doubt that the anal fin is of prime importance in clasping.

TABLE II.

(*F. heteroclitus* males, collected May 14, 1907.)

No. of Specimens.	Distribution of Contact Organs.
3	On anal fin only.
2	On anal fins and on the scales of the body zone between the dorsal and anal fins.
5	None.

Specimens taken in large numbers about two weeks later nearly all showed the full equipment of contact organs. The development, therefore, is a very rapid one, taking place largely within a fortnight.

The conditions in the other species (*F. majalis*) were somewhat different. Specimens collected April 27, 1907, showed no contact organs. Those collected on May 14 of the same year showed conditions tabulated as follows:

TABLE III.

(*F. majalis* males collected May 14, 1907.)

No. of Specimens.	Distribution of Contact Organs.
2	Fairly numerous on the cheeks.
4	On cheeks, top of head, and around eyes.
5	On cheeks, top of head, around eyes, and a few rudiments on the body zone between dorsal and anal fins.
1	Rudiments on cheeks and around the eyes, a few on the tips of the anterior rays of the dorsal fin.
1	Fairly numerous on cheeks and around the eyes, scattering on top of head, a few on the dorsal fin, and numerous on the body zone between the dorsal and anal fin.
1	Numerous on cheeks and around the eyes, scattering on top of the head, numerous on body zone between the dorsal and anal fins, and on the distal portions of nearly all of the rays of the dorsal fin, and a few on the posterior lobe of the anal fin near the tips of the rays.

The order of appearance of the organs in the various regions seems to be about as follows: (1) On cheeks and around the eyes, (2) on top of head, (3) on the body zone between the dorsal and anal fin, (4) on the dorsal fin, (5) on the anal fin.

Any attempt to correlate this order of appearance with the relative functional importance of the organs in the different regions meets at once with serious difficulties. It can hardly be supposed that the organs in the head region are of more importance as spawning accessories than are those on the dorsal fin. The fact, however, that the organs on the head are used in the preliminary phases of spawning, such as courtship and rivalry, might better account for their early appearance. Thus the factor of priority of functioning might be conceived of as outweighing functional importance. In *F. heteroclitus*, however, the opposite condition seems to prevail. The difference may lie in the fact that, in *F. heteroclitus* the importance of the fins as claspers is much more pronounced than in *F. majalis*.

The order of appearance of the individual contact organs on the fin rays has been referred to. They appear first on the distal portion of each ray, but, as the ray increases in length, those first formed are separated farther and farther from the tip of the ray. Later they appear proximal to those that were first formed.

On the scales the first organ to appear is always located in the center of the exposed arc of the scale. Many specimens of both species taken in April and in May showed, in certain regions, but one contact organ to the scale. From the center they are produced outward in both directions. A common condition observed in specimens of *F. heteroclitus* taken in May was that of one rather large organ in the middle of the free edge, flanked on each side of the center by a small organ. There is abundant evidence that other organs may later be intercalated between those first formed.

The contact organs disappear for the season in about six to eight weeks after the close of the spawning season. Specimens collected early in September showed only the faintest traces of the organs. Evidently, judging by appearances of these vestiges, they are partly worn off by friction and partly resorbed. They leave a permanent record of themselves in the angularly wavy outline of the newest osseous ring of the scales. No such trace seems to be left on the fin rays.

Function. — Two possible functions might be subserved by the contact organs, that of frictional surface on the parts of the body of the male with which he holds the female in spawning, and that of contact sensation. That the former function is well subserved is evidenced by the roughness that one notes with the fingers wherever the contact organs are well developed and numerous, and by the fact that the male is actually able to hold the female quite firmly for several seconds. The fighting males are able to inflict any real injury upon one another by means of the contact organs is highly improbable, although they strike one another fiercely with parts well armed with the latter. That scales and fins are decidedly sensitive to contact is well known. Microscopic examination of these parts, prepared by special neurological methods, failed to show that the contact organs were any more richly enervated than the rest of the free edge of the scale or the general surface of the fin. It is probably true, however, that the projecting position of the contact organs renders them more subject to contact sensation than the surrounding flat regions of scales and fins.

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BIOLOGICAL BULLETIN

A CASE OF NORMAL IDENTICAL QUADRUPLETS IN THE NINE-BANDED ARMADILLO, AND ITS BEARING ON THE PROBLEMS OF IDENTICAL TWINS AND OF SEX DETERMINATION.[1]

H. H. NEWMAN AND J. THOS. PATTERSON.

In a former contribution from this laboratory by A. M. Spurgin, M.D., occurs the following statement: "A year ago, Dr. W. M. Wheeler, of the School of Zoölogy, had the good fortune to secure four embryos of the *Dasypus novemcinctus* from an adult female which had been kept in the laboratory for several weeks. . . . He found four placentæ inclosed in one amnion, but has not since had time to study the subject further."

When the writers arrived in Austin last September they made arrangements to obtain material with which further to investigate this suggestive problem, and were fortunate enough to enlist the services of a naturalist living in a part of Texas where the armadillo abounds. In this way we have been able to secure several gravid females, from a study of which a number of interesting facts have been brought to light.

There are always four embryos, corresponding in number to the two pairs of mammæ, which are thoracic and abdominal in position. The embryos are not enclosed in one amnion, but each has its own distinct amniotic envelope, the cavity of which does not communicate with those of the adjacent amnia. This was proved by the experiment of inflating one amnion and noting that the air did not pass to the cavities of the contiguous amnia. The four amnia, however, are enclosed in a single chorionic vesicle.

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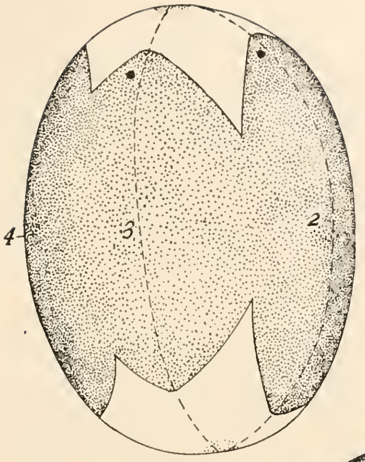
[1] Contribution from the Zoölogical Laboratory of the University of Texas, No. 98.

[2] *American Journal of Anatomy*, Vol. III., No. 1.

An examination of the external villous layer of the chorion shows that placentation is unique in that areas of villi, although apparently forming a complete zone about the equatorial region of the uterine wall, are in reality arranged in four closely approximated ovoid areas, two large and lateral in position, and two smaller, one dorsal and the other ventral. The proximal (*i. e.*, the posterior or vaginal) and distal ends of the chorionic vesicle are practically free of villi, except at each pole, where the presence of a small tuft of villi causes the chorion to adhere very firmly to the uterine wall (see Fig. 1). The villous zone, composed as it is of four ovoid areas, extends into the polar regions in the form of four scallops at each end, and on the amniotic side of the four scallops are situated the points of attachment of the four umbilical cords (see Fig. 2).

In the advanced stages of gestation the chorionic vesicle is shaped very much like a football, and the four lines on the inside wall, along which the adjacent amnia meet, correspond to the seams of the ball (see Fig. 1). In the young stages, however, the chorion is in the shape of an octaedron with rounded edges, and flattened dorso-ventrally, and has its entire surface covered with villi (see Fig. 3). From this we may conclude that the definitive condition of the placenta has been attained by a localization of the villi into ovoid areas, each of which is to be looked upon as the vascular center for an umbilicus.

The points of attachment of the umbilical cords lie close to the amniotic partitions, and when the chorion is viewed from the distal end the cord in each case is seen to be situated just to the right of the partition (see Fig. 2). In embryos about one fourth grown the partition between any two contiguous embryos may be easily separated into its component parts, but in older stages the fusion of the membranes is so complete that separation is impossible. In late stages, therefore, the chorionic cavity is completely divided longitudinally into four separate compartments, each of which contains an embryo. Although the portions of the chorionic wall that lie between the successive amniotic partitions are in the form of quadrants, yet they do not coincide with the ovoid areas; for the partitions do not meet the chorionic wall in lines corresponding to the divisions of the ovoid



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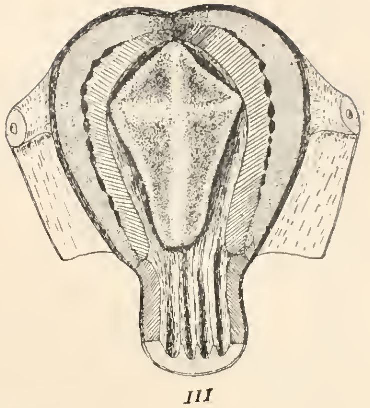

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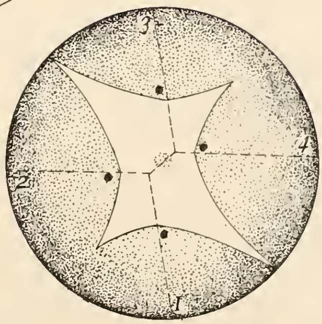

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Fig. 1. A diagrammatic representation of an approximately dorsal view of a chorionic vesicle taken from the uterus about two weeks (estimated) before the young would have been born. Three of the ovoid areas are in view, and the broken lines represent the lines along which the amniotic partitions meet the inside wall of the chorion. The clear areas at the ends are broken into by the scallops of the ovoid areas. In this, as in the succeeding figure, the points of attachment of the umbilical cords are indicated by large dots. Note especially that the indentations between the scallops of areas 3 and 4 are much shallower than those between areas 2 and 3, leaving a broader connection between the former than between the latter. This is an indication that embryos 3 and 4, located respectively on dorsal and left lateral areas (similarly embryos 1 and 2, located on the ventral and right lateral areas), are natural pairs. For the significance of this arrangement see table and text. One half natural size.

Fig. 2. A view of the distal end of the preceding figure. This is introduced to show the relation existing between the amniotic partitions within the chorionic cavity. The figure also brings out the fact that the embryos may be paired, together with the ovoid areas to which they are attached by the umbilical cords. One half natural size.

Fig. 3. This shows a chorionic vesicle *in situ*, as revealed by splitting open the uterus along the mid-ventral line. The age of this vesicle is estimated at one month. Unfortunately the splitting was done before the specimen reached our hands, and extended so deep as to divide the vesicle into two parts. The parts, however, were well preserved *in situ*, and the reconstruction could be made with certainty. Note that the vesicle is octaedronal in shape, and that its entire surface is covered with villi. Natural size.

areas, but each partition passes through the middle of an area and thus intersects the apices of the proximal and distal scallops (see Fig. 1). The four partitions, furthermore, do not meet within the cavity in a line corresponding to the polar axis of the vesicle, but are fused in such a way that the cavities containing the embryos numbered 1 and 3 meet on their inner faces, while those containing 2 and 4 do not (see Fig. 2).

In a note in the current issue of *Science* (April 30) Professor H. H. Lane gives a brief account of placentation in the armadillo that differs in a number of particulars from the above. The discrepancies might be explained by the fact that Lane's account rests upon observations made upon a single deciduate placenta, taken after the birth of a litter. In addition to its incompleteness, his account differs from that offered here chiefly in locating the points of attachment of two of the umbilical cords on each of the large "disc-shaped areas," and none on the smaller areas. This condition is certainly not found in any of our material. A more fundamental difference between the two accounts lies in the interpretation of the condition of the chorion, Lane holding an opinion completely out of accord with the facts here presented, that the definitive chorion is the product of a complete fusion of four chorionic vesicles into one.

The unique interest of this case lies in the fact that the four embryos of a given set are not only invariably of the same sex, but are practically identical in many other respects. Measurements of the body and head lengths showed no essential differences between the individuals of a set of quadruplets, but a more searching comparison, consisting of carefully confirmed counts of the total number of plates in the nine bands of the armor, revealed, as one might expect, slight departures from complete identity. The results obtained from the examination of two sets will serve to indicate the small range of these departures.

Set A (Females).		Set B (Males).	
No. 1	556 (+1) plates[1]	No. 1	571 (+2) plates
No. 2	555 (+2) "	No. 2	573 (+1) "
No. 3	553 (+2) "	No. 3	569 "
No. 4	551 (+4) "	No. 4	568 "

[1] The numbers enclosed in parentheses refer to certain rudimentary plates that are more or less united with other plates. It is impossible to tell in the embryos whether

In both cases the range of variation is five, or less than one per cent. The difference in totals between the two sets is very much greater — a difference that does not indicate a sexual dimorphism, for an examination of five unrelated specimens of both sexes that happen to be on hand, shows a range of from 547 to 575 plates, a variation nearly six times as great as that existing between a given set of quadruplets.

This very marked similarity among the individuals of a given set suggests that we have here a condition showing a degree of identity that, in all probability, is at least as close as that found in the well-known case of "true" or "identical twins" in the human race, where it is supposed that each embryo arises from from one of the two blastomeres of the two-cell stage. In the case of *Dasypus*, each embryo probably arises from one of the blastomeres of the four-cell stage.

This immediately turns our attention to a further consideration of the fœtal membranes, the amnion and the chorion, and of the manner in which they arise. It was stated above that each embryo is enclosed in a separate amnion, and that the four amnia are within a single chorion. We say single chorion advisedly, because its surface gives no indication of being a multiple structure, that is, the product of the fusion of four chorionic vesicles at an earlier period, as suggested by Lane; nor do the facts revealed by a study of sections of the chorionic wall seem to bear out such an interpretation. One must admit, however, the great difficulty of any attempt at a correct interpretation of the exact relationship existing between the fœtal membranes as seen in these advanced stages. The solution of that problem must be sought in a study of young stages, when the membranes are in process of formation.

To have four ova ripen, be fertilized, and reach the uterus at the same time, and always arrange themselves in a definite fashion

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or not these small plates would later have become separated off to form distinct ones, but we are inclined to believe that such is the case, for in the adult armor there appear to be no signs of double plates. If we count these rudimentary plates as complete plates, then embryos 1 and 2 (Set A) would have exactly the same number of plates, as would also embryos 3 and 4. The numbers of plates in the corresponding embryos of Set B, although not identical, differ to the minimum extent, one plate in each case.

with reference to one another and to the uterine wall, would be in itself a remarkable coincidence. The chief difficulty, however, in the way of accepting the view that the chorionic vesicle is a multiple structure lies not so much in the interpretation of the relation existing between the fœtal membranes, nor in the discovery of the mechanics involved in the attainment of such a relation, as in the explanation of the fact that the embryos of any given set are always practically identical and of the same sex. At first sight it might seem that this identity could be explained by the fact that the embryos are apparently under the same environmental influences, inasmuch as they are enclosed within a single chorion. But in reality they are not under exactly the same influences, for each embryo has its own amnion, and is, therefore, surrounded by a fluid apparently as distinct as though each were in a separate chorion. This forces us to the conviction that the four embryos of a set are derived from a single egg.

If this is the case, one can only conjecture as to the manner in which the conditions seen in the older stages have arisen, and any suggestions which we may have to offer at this time must be taken as tentative. It may be supposed, for example, that the developing egg, after it has reached a stage corresponding to that of an inner-cell mass and trophoblast in other mammals, has, in the case of *Dasypus*, four inner-cell masses — a cell mass for each quadrant; and furthermore that the cells of any given inner-cell mass, together with the trophoblastic cells of its quadrant, are the lineal descendants of one of the blastomeres of the four-cell stage. This possible interpretation receives a striking confirmation in the fact that the four embryos can be arranged into two pairs, the individuals of which approach almost complete identity; and these identicals are not only adjacent to each other, but are also attached to placental areas that are closely united (see Figs. 1 and 2 and table). If all four embryos are derived from a single egg, this is exactly what we should expect to find; for surely the two individuals derived from one of the blastomeres of the two-cell stage ought to be more nearly similar to each other than to the individuals of the other blastomere. If the above be granted, it is an easy matter to explain the origin of the conditions found in late stages; and the condition of a single chorion with four amnia would receive a rational explanation.

We are, of course, aware that other possible interpretations might be given, such as has been offered from time to time to account for human identical twins. Our studies of the ovarian tissues of *Dasypus* have excluded the most plausible of these, viz., that the ovum may have more than one germinal vesicle. Further investigation will have to settle the next most plausible explanation offered, that the polar bodies may become functional. None of the other suggestions found in the literature seems as credible as the view to which we incline, and in support of our contention we have facts derived from an examination of two sets of ovaries from gravid females. In one set there is but a single corpus luteum, and, while in the other there are two corpora lutea, one has practically disappeared, and evidently had been formed during a previous pregnancy.

After all, the development of the fœtal membranes is a matter of secondary interest, as compared with the more fundamental problem of the identity of the embryos of a set, and its corollary that of sex determination. The bearing of this work on the latter problem is obvious, and we hope that a study of the early developmental stages will lend a solution to this problem, and also furnish a satisfactory explanation of the puzzling question of "identical twins"; and thus raise this explanation from the plane of conjecture to the dignity of observed fact.

Austin, Texas,
April 30, 1909.

THE GENERAL BIOLOGICAL SIGNIFICANCE OF CHANGES IN THE PERMEABILITY OF THE SURFACE LAYER OR PLASMA-MEMBRANE OF LIVING CELLS.[1]

RALPH S. LILLIE.

The osmotic properties of living cells have been the subject of repeated investigation since the fundamental experiments of Pfeffer and de Vries, and especially since the rise of the theory of osmotic pressure and the extension of the gas laws to dissolved substances by van't Hoff in 1887.[2] One remarkable result, of the highest significance for general physiology, has been perhaps the main outcome of these researches; living cells of the most diverse kinds have been found to possess in common a high degree of physical impermeability to most of the diffusible non-colloid substances normally occurring in the cells and in their surroundings. The surface film or so called plasma-membrane of cells, while readily permeable to water, offers a highly efficient barrier — at least during the resting or unstimulated state — to the entrance or exit of many dissolved substances of relatively low molecular weight, even of those normally present in protoplasm, as sugars, amino-acids, and neutral salts. This impermeability is a property of the normal living cell, unmodified by experimental conditions. A direct proof of this is the characteristic turgor of plant cells, which is due to the osmotic pressure of crystalloid substances dissolved in the cell-sap; it is clear that the osmotically active substances — sugars, organic acids, and other relatively simple compounds — are ordinarily unable to traverse the limiting surfaces of the protoplasm; were they able to do so the maintenance of the high internal pressures known to exist would be impossible. Such impermeability is a peculiarity of the *living* cell and disappears at death, which is always associated with a loss of turgor and a diffusion of dissolved materials (coloring matters, etc.) from the cell. The increase in permeability may be the consequence, or it may be the cause, of the death-process; at all events it is the invariable concomitant of the latter — a fact apparently signifying that the living condition is incompatible with more than a temporary loss of impermeability.

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[1] From the Physiological Laboratory, Department of Zoölogy, University of Pennsylvania.

[2] A full account of the earlier researches will be found in Hamburger's "Osmotischer Druck und Ionenlehre in den medizinischen Wissenschaften," Wiesbaden, I. F. Bergmann, 1902–4. See also Höber's "Physikalische Chemie der Zelle und der Gewebe, 2d ed., Leipzig, Engelmann, 1906.

The dependence of certain fundamental life processes (as growth, which is a manifestation of osmotic energy) on the impermeability of the plasma-membrane is more clearly apparent in plants than in animals. The proof that a similar impermeability characterizes animal cells is due mainly to the use of the plasmolytic method, which was first accurately applied to plants by de Vries, and has later been extended to animal cells by Hamburger, Overton, Koeppe and others. The principle of the method is well known; the turgor-pressure of plant cells, due to the osmotic pressure of the cell-contents, may be compensated by the application of an external osmotic pressure, *provided* that the boundary surface of the protoplast is impermeable also to the external solute; over-compensation, with compression of water from the cell and a shrinkage of the protoplast from the cellulose cell-wall, results if the external pressure distinctly exceeds that of the cell-contents; under-compensation leads to absorption of water; while with equality of external and internal osmotic pressures no osmotic change is seen (cases of hypertonic, hypotonic and isotonic solutions respectively). A means is thus afforded of testing the permeability of the plasma-membrane to different substances. If, using a moderately hypertonic solution of a given substance, a loss of water results which remains permanent during the period of immersion in the solution, impermeability relatively to that substance is indicated (the case of sugars, polyhydric alcohols, neutral salts). If the cell loses water only temporarily, afterwards regaining its original proportions, or even swelling still further, *gradual* entrance of the substance into the cell is indicated; such effects usually appear with solutions of urea, glycerine, or glycol, to which most cells are slowly permeable. If no plasmolysis results, even with strongly hypertonic solutions, a free permeability to the dissolved substance must exist; hence such solutions typically induce swelling of cells; many organic substances, particularly fat-solvents, belong to this group. Another method of testing permeability has been employed by Hedin, which has the advantage of permitting of quantitative application. A known quantity of the substance to be tested is added to a given volume of a somewhat dense suspension of the cells studied (chiefly blood corpuscles). After an interval the mixture is centrifuged and the partition of the added substance between cells and suspension-medium may then be determined cryoscopically. The results gained by these two methods agree closely. The permeability of animal cells has been studied in considerable detail by various modifications of these methods. Overton in particular has compared the respective permeabilities of animal and plant cells and has shown that essentially the same relations hold for both. The conditions are highly constant and characteristic. All the cells examined have proved impermeable to sugars, polyhydric alcohols (erythrite and higher), amino-acids (glycocoll, alanin, leucin, asparagin, taurin), and most neutral salts of alkali and alkali earth metals; difficultly permeable to dihydric alcohols, urea and glycerine; and freely permeable to monohydric alcohols, ethers, esters, aldehydes, normal and substituted hydrocarbons — in general to such substances as exhibit in common the property of dissolving fats or of being dissolved in them. In applying such methods it must be borne in mind that many substances, including particularly the last named group, alter the normal permeability of the plasma-membrane; allowance has therefore to be made for changes in the normal permeability due to the direct action of the substances investigated. Most of the substances of the first and second of the above groups have, however, relatively slight action of this kind; and impermeability or difficult permeability to these substances appears to be a very general if not a universal rule for both plant and animal cells.

Impermeability to sugars, polyhydric alcohols, amino-acids, neutral salts, combined with ready permeability to those various organic substances which have in common fat-dissolving or lipolytic properties, appears thus to be a fundamental characteristic of living cells. The view that the surface layer of protoplasm is fatty in nature, early expressed by Quincke and others, has thus been confirmed by a study of the permeability, since fat-solvents will readily enter and traverse a layer consisting largely of fatty substances. Overton has shown that the permeability of cells is in general such as would be expected if the plasma-membrane consisted largely of lecithin and cholesterin, the chief members of the so-called lipoid group of substances, which appear to be constant constituents of protoplasm. He found that those dyes which readily enter cells (*intra vitam* dyes) are very generally soluble in lipoid mixtures or in organic solvents containing lipoids — even if insoluble in triolein and other simple glycerides — while the group of non-vital dyes, particularly the sulphonic acid derivatives, are insoluble in such solvents.[1] A relation between lipoid-solubility and ability to enter cells thus exists for dyes, according to Overton. The rule that such solubility implies ready penetration of a dye into a cell and *vice versa* appears, however, not to be without exceptions;[2] the matter is complicated by the readiness with which the normal permeability may be modified by the very presence of the substance whose penetration is in question. There are, however, other facts which indicate that the distinctive vital permeability of cells is intimately related to the presence of these substances. Chief among these are the peculiar relations of lecithin and cholesterin to hæmolysis, a change which seems to depend primarily on alteration of the permeability of the surface layer of the erythrocytes; the action of various hæmolytic substances (saponin, agaricin, solanin, cobra poison, tetanolysin) is furthered or checked in a characteristic manner by the addition of lipoids to the blood serum. Again, the observations of Pascucci[3] in Hofmeister's laboratory on the permeability of artificial membranes impregnated with lipoids, and on the composition of the stroma of blood corpuscles, afford strong support to the view that lipoids play an essential part in the formation of the plasma-membrane. Naturally the latter is not to be regarded as a simple layer of lipoids; it appears rather to be a surface film of highly complex composition, probably consisting of a mixture of substances, chiefly protein but largely lipoid, that have collected at the surface in consequence of their effect in lowering surface-tension. Numerous observations have shown that coherent films may thus be formed at the boundary surface between two fluids.[1] The phenomenon is now recognized as a special instance of the operation of a principle first defined by Willard Gibbs: conditions of equilibrium require that substances which lower the surface-tension of a solvent should collect in the surface layers in higher concentration than in the interior. Proteins and lipoids show this behavior. It must be granted, however, that the conditions which render the plasma-membrane of resting cells so peculiarly impermeable to soluble substances — provided these do not alter its chemical composition or colloidal consistency — are imperfectly understood. The facts of electrical stimulation indicate that the characteristic electrically polarized condition or physiological polarization of the plasma-membrane is a factor of fundamental importance.

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[1] Overton, "Jahrbücher für wissenschaftliche Botanik," 34, 1900, p. 669.

[2] Cf. the critique of Brailsford Robertson, *Journal of Biological Chemistry*, 1908, vol. 4, p. 1. Robertson nevertheless regards the surface film as partly composed of lipoids, although he ascribes more importance to the surface film of modified protein.

[3] Pascucci, *Hofmeister's Beiträge*, 1905, 6, pp. 543, 552.

During the greater part of its existence the cell thus appears almost completely shut off from osmotic or diffusive exchange with its surroundings. It is evident, however, that this impermeability to the food-materials and to the salts of protoplasm cannot exist at all times and under all conditions; such substances do enter the cell, and since such entrance implies penetration of the surface layer, the latter must under certain conditions become permeable. A specifically physiological problem is here encountered; either soluble food substances reach the interior of the cell quite otherwise than by a simple process of diffusion, or the permeability of the plasma-membrane must at certain times undergo functional alterations so as then to admit such substances. There is in fact evidence that the permeability of the plasma-membrane does increase at times, *e. g.*, under the influence of carbon dioxide, or during stimulation; and it is possible that food materials may enter chiefly or exclusively at such times. Nevertheless, the whole question as to the mode of entrance of soluble food materials into cells still presents many difficulties.

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[1] Cf. Ramsden, *Zeitschrift für physikalische Chemie*, 1904, vol. 47, p. 336. Metcalf, *ibid.*, 1905, vol. 52, p. 1. Literature and review in this paper.

The process probably requires the performance of work on the part of cells, just as does the separation of a secretion by a gland. The kidney performs osmotic work in concentrating its secretion, and the cells of the intestinal mucosa absorb many substances against concentration gradients, a capability also implying performance of work. The rapidity with which salts and food-materials are absorbed seems indeed incompatible with the existence of an impermeable plasma-membrane; yet whenever resting cells are tested with regard to their physical permeability the above unequivocal results appear. Evidently the decisive factors in absorption as also in secretion are largely independent of physical diffusion.[1]

Whatever the actual case may be — and the problem remains unsolved for the present — there is no doubt that impermeability to the diffusion of many dissolved substances of low molecular weight is a highly distinctive and even necessary characteristic of living cells. The following considerations will make this clear. Substances of relatively low molecular weight and high diffusibility form an important part of the living protoplasmic complex. In the specific metabolism of any animal the protein and carbohydrate food materials are split respectively to amino-acids and sugars, both highly diffusible substances; and many other diffusible products important in metabolism are formed by oxidation or hydrolysis. These substances must not be lost from the cell. It is plain that any specific organism must exhibit a constant and specific metabolism — is indeed the product or the manifestation of this. Now the existence of specific metabolic processes in any cell requires the presence of many interacting substances in proportions that must not vary widely from a constant mean; in other words, constancy in the character of its metabolic processes is essential to the specificity of a particular cell. Its protoplasm may be regarded as a mixture of diverse yet constantly present substances in an approximate chemical equilibrium of a highly complex order. Any such constancy of composition, implying constancy in the conditions of equilibrium, would be impossible in a system not very completely isolated from its surroundings. In the cell this isolation is due to the presence of an impermeable surface film. A marked degree of impermeability to the great majority of its diffusible constituents is thus indispensable to the continued existence of a highly complex heterogeneous system like the cell. To certain substances like oxygen and carbon dioxide the plasma-membrane appears quite freely permeable, though even in the case of these substances the permeability seems to differ greatly at different times.[1] In general the conclusion seems justified that the presence of this impermeable boundary is an indispensable condition of the most fundamental chemical characteristics of living matter. We may say indeed that just as the high development of chemistry would have been impossible without the use of vessels in which the interacting substances could be confined and isolated from the surroundings, so the development in phylogenesis of the complex chemical specificity of living organisms has depended on the isolation of the protoplasmic chemical system from its surroundings by a physically impermeable boundary. The impermeability of the plasma membrane thus appears not as a merely incidental character of living cells, but as a primary condition both of their development and of their continued existence.

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[1] Cf. Asher, *Biochemische Zeitschrift*, 1908, XIV., p. 1, "Untersuchungen über die physiologische Permeabilität der Zellen."

The non-permeability of cells to many electrolytes is an especially significant characteristic. Neutral salts of the alkali and alkali earth metals are almost invariable constituents of protoplasm; yet the plasma-membrane, as the investigations of Overton and others have shown, is impermeable or difficultly permeable to many if not all of these. This implies impermeability to the undissociated molecules and to one or other or both of the two classes of ions resulting from dissociation. If a membrane is partially permeable to both ions of an electrolyte the chances are that it will be unequally permeable to these; and the case is conceivable that it should be freely permeable to one ion but not to the other. Ostwald[2] in 1890 first directed attention to this possibility; such a membrane, if interposed between two unequally concentrated solutions of the electrolyte, would then be the seat of an electrical potential difference, since there would be a separation of electricities due to the passage of a certain small proportion of ions of one sign through the membrane; this would continue until the electrostatic tension thus arising balanced the osmotic pressure difference between the ions on opposite sides of the membrane, when the ionic transfer would cease. A certain potential difference — constant (at a given temperature) with unchanged permeability of the membrane and concentrations of the electrolyte — would then exist between opposite surfaces of the membrane. The presence of membranes having such a differential permeability in reference to the anions and cations of the tissues might, Ostwald pointed out, be the source of the electrical phenomena of living organisms; and this suggestion has been further developed by Bernstein, followed by Brünings, Hoeber and others. The theory based on this interpretation — the so-called "membrane theory" of the origin of the bio-electric currents — appears to-day as the most adequate explanation of these complex and puzzling phenomena. Its merit consists mainly in the introduction of a third variable — in addition to character and concentration of the ions at the boundary surface[1] — namely, the ionic permeability of the membrane itself. This is subject to more or less sudden alteration either in the direction of increase or decrease; and the sudden and pronounced electrical changes accompanying stimulation and inhibition, as well as the remarkably high potential differences found in some instances (electric fishes), become readily intelligible on this theory.

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[1] The permeability to fat-solvents is an incidental, not a vital, peculiarity, since these substances are not normally present in protoplasm or its surroundings.

[2] *Zeitschrift für physikalische Chemie*, 6, 1890, p. 71.

The outer uninjured surface of a resting cell — *e. g.*, a muscle cell[2] — always proves positive relatively to the interior whenever the latter is exposed by any kind of injury or by chemical or other alteration of the surface. The membrane theory therefore assumes that the plasma-membrane is freely permeable during rest to the cations of a certain electrolyte or electrolytes contained within the cell, but not to its anions. This condition may, however, undergo temporary alteration, as during stimulation, when there is invariably a fall in the potential difference between exterior and interior of the cell, such as would result from an increased permeability to anions. During life such potential changes occur only temporarily; they are an apparently inseparable accompaniment of any form of stimulation. On the death of the cell, however, there follows a marked and permanent increase in the general permeability, and this change is always associated with a permanent fall in the potential difference between surface and interior. That the normal potential difference observed during life is correlated with the normal impermeability of the plasma-membrane thus appears highly probable. The plasma-membrane is hence to be regarded as the seat, during life, of a permanent electrical polarization which is diminished during stimulation, and disappears or is greatly diminished at death, when the vital semi-permeability is lost. The cation to whose penetration the external positivity is due was at first supposed to be potassium, which is present in relatively large proportion in the interior of vertebrate muscle cells; but this is now known not to be the case. Were it so stimulation would involve a loss of potassium salts from the cell, of which there is no evidence; moreover Hoeber has shown that increasing the external concentration of potassium ions does not reverse the direction of the demarcation current. The indications point strongly to the rapid and highly penetrating hydrogen ion as the source of the outer positive potential of cells; and since acids, particularly carbonic acid, are known to be formed in metabolism and to leave the cell during stimulation, there exists in fact within the cell a constant source of hydrogen ions to account for the characteristic surface polarization. That such a system should show a surface polarization of the above kind is, in fact, not surprising. The depolarization associated with stimulation and with the death process implies the loss of the polarizing electrolyte from the cell; it has long been known that carbonic and possibly other acids are evolved at such times in greatly increased quantity.

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[1] Assuming constant temperature.

[2] Of course observation cannot be directly made on single cells — unless on certain egg-cells — a possibility exemplified by Miss Hyde's investigations cited below. The above statement assumes that what is observed for parallel bundles of cells, *e.g.*, such a muscle as the frog's sartorius, is true for single components of the bundle.

Proofs that the surface layer of cells is unequally permeable to the anions and cations of many electrolytes have been furnished by the investigations of Hamburger, Koeppe, and Höber.[1]

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[1] An account of these investigations is given in Höber's "Physikalische Chemie der Zelle," pp. 303 *seq.*

These have yielded the result that under the influence of carbonic or other weak acids (*i. e.*, of H-ions in low concentration) blood corpuscles give evidence — by showing an exchange of anions with the surroundings or by characteristic behavior in electrical convection — of becoming much more freely permeable to various anions (Cl, Br, SO_4, HCO_3, NO_3) than before, while the permeability to cations appears unchanged. This increase in permeability to anions is readily reversible on removing the carbon dioxide. Proof is thus afforded that the permeability of cells to ions is a variable quantity, which may undergo increase or decrease according to circumstances. The theoretical importance of a demonstration of reversible changes in ionic permeability is evident when we consider the essential rôle which, on the membrane theory, such changes play in stimulation.

The permeability of the living cell is thus not a constant factor, but one subject to alteration under a variety of conditions. The reversible changes induced by stimulating or inhibiting agencies, whose respective action appears to consist mainly in increasing or decreasing the normal permeability, are of particular physiological interest. Alterations of permeability are also induced by a large class of foreign substances which act directly upon the plasma-membrane. Such are in general: (1) The class of lipolytic substances or organic fat-solvents; these directly affect the lipoids of the plasma-membrane; if present in sufficient concentration they produce marked increase in the permeability of the surface layer of cells. The effect is conspicuous in pigment-containing cells (erythrocytes, egg-cells, etc.); the pigment leaves the cells and colors the solution (hæmolytic action of fat-solvents). Increase of permeability sufficient to allow the exit of colloid substances is usually irreversible and destructive to the cell. (2) Various electrolytes; these affect primarily the aggregation-state of the colloids. Neutral salts of alkali and alkali earth metals produce largely *reversible* changes in permeability; with salts of heavy metals, and with acids and alkalis above low concentrations the effects are irreversible, hence (in part at least) the greater toxicity of this class of electrolytes; alkalis may have also a saponifying action. (3) A large and miscellaneous class of poisonous substances — cytotoxins, hæmolysins, alkaloids and glucosides of various kinds (saponin, solanin, agaricin, quillain, etc.); these increase the permeability of blood corpuscles, liberating hæmoglobin and eventually destroying the cell. Such substances alter the plasma-membrane partly by their action on the lipoids (lipoid-soluble alkaloids), partly by some specific action on the proteins, as apparently in the case of foreign blood sera. (4) Photodynamic substances (eosin, fluorescein, chlorophyll,[1] etc.); these also appear to act in the light primarily on the plasma-membrane, increasing its permeability. Tappeiner's observations[2] indicate clearly that eosin solutions first effect the peripheral layers of the cell. Paramæcia exposed to solutions of the dye in the dark, washed free, and then exposed to the light are but little affected; blood corpuscles show a certain difference in behavior and allow some entrance of the dye in the dark, but hæmolysis is much more rapid if the corpuscles are illuminated while in the solution, than if, after equally prolonged treatment in the dark, they are transferred to an eosin-free medium and then illuminated: *i. e.*, the effect is largely if not exclusively dependent on a surface action. Harzbecker and Jodlbauer[3] find the same general result. Microscopical observation also indicates that the initial stage of the destructive action consists in a disturbance of the osmotic equilibrium between corpuscle and medium, such as would result if the semi-permeability of the plasma-membrane were abolished. The corpuscles first swell, indicating entrance of water; this occurs before any perceptible entrance of the dye. P. v. Baumgarten[4] finds the same initial change in the action of hæmolytic substances, and has referred hæmolysis primarily to a loss of osmotic equilibrium consequent on alteration of the surface layer. This view is also upheld by Hamburger.[5] The increase in permeability, in addition to destroying the osmotic equilibrium, will naturally further the entrance of the toxic substance and the action will then affect the entire cell. In general toxic action must be regarded as depending in many cases primarily on an alteration, particularly an *increase*, in the normal permeability of the cells.

[1] Hausmann, *Biochemische Zeitschrift*, 1909, XVI., p. 294.

[2] Tappeiner, *Biochemische Zeitschrift*, 1908, XII., p. 290; *ibid.*, XIII., p. 1.

[3] Harzbecker u. Jodlbauer, *Biochemische Zeitschrift*, 1908, XII., p. 306.

[4] P. v. Baumgarten, *ibid.*, 1908, XI., p. 21.

[5] Hamburger, *loc. cit.*, vol. 3, p. 360.

Hence the hæmolytic action of many toxins: the injury to the plasma-membranes is naturally not confined to the blood corpuscles — the effect is merely most plainly evident in these cells; but all or most of the cells of the organism are to be regarded as similarly affected.

The above substances produce irreversible or toxic changes in cells by influencing through their chemical or solvent or coagulative action the permeability of the plasma-membrane. Essentially similar effects may result in many cases from moderate rise of temperature. Thus exposure of frog's muscle to temperatures approaching 40° for a short time produces the typical phenomenon known as heat-rigor, in which the muscle shortens and thickens while its substance undergoes complete coagulation and acquires a pronounced acid reaction. A closely similar change results when a muscle is immersed in a saturated solution of a lipoid-solvent like chloroform in an indifferent medium (*e. g.*, Ringer's solution). In both cases the changes in the muscle are associated with a loss of semi-permeability and of electrical polarization; and they are almost undoubtedly direct consequences of this since neither the moderate rise of temperature nor the presence of chloroform has by itself any such coagulative effect on the colloids composing the muscle substance; while loss of semi-permeability, with consequent disturbance of chemical equilibrium and production of acids, may readily produce just such effects.[1] Why such moderate heat should so profoundly alter the properties of the plasma-membrane is not evident at first sight; possibly the colloidal aggregation-state is the condition immediately influenced by the change of temperature; possibly the primary effect is a chemical one.

The above are instances of artificially produced or pathological changes in permeability. Normal or functional changes in this property appear to be of frequent occurrence in living organisms. Thus there is highly conclusive evidence that the general process of stimulation is dependent on a temporary and reversible increase in permeability. Very clear indications of this kind are seen in motile plant organs. The sudden movements of Mimosa leaves, for example, result from a loss of turgor of the pulvinus cells, due to escape of cell-sap — a process which, as Sachs[1] long ago pointed out, signifies temporary increase in permeability. Since the movement is induced by the same stimuli as cause contractions in muscle-cells, and since it is accompanied by a similar electrical variation or action-current (as Burdon-Sanderson first showed), the inference seems natural that the primary change in the stimulation of animal cells is also a temporary increase in permeability. It remains therefore to inquire how such a change could lead to such seemingly disproportionate liberation of chemical and mechanical energy. I have recently discussed the relation of permeability-changes to stimulation in some detail and need not repeat *in extenso* the facts and arguments presented in these papers.[2] Briefly these are as follows: (1) Artificially-induced increase in permeability through the action of various electrolytes, lipoid-solvents, or protoplasmic poisons, produces contraction in muscle cells; (2) the death change, which is associated with an increase in permeability, produces the same effect; (3) the electrical change always associated with stimulation is of a kind indicating temporary depolarization of the plasma-membrane; (4) the loss of irritability at the height of stimulation (refractory period) is what should be expected if the plasma-membrane becomes freely permeable to ions at such times; (5) there are certain inorganic phenomena, showing remarkably close analogies to stimulation, where the effect depends on dissolution of a surface-film (pulsatile mercury and hydrogen peroxide catalysis of Bredig and his pupils); finally (6) the chemical effects of stimulation — increased production of carbon dioxide with increased acidity of the muscle substance, etc.— receive a consistent explanation on physico-chemical grounds if it is assumed that with the increase in permeability the resistance to the escape of the products of oxidation — particularly carbon dioxide — and hence also to the progress of the oxidative energy-yielding reaction is suddenly diminished; the velocity of this reaction hence undergoes a corresponding sudden increase. Inhibition is to be regarded as the inverse of stimulation and as dependent on still further decrease of the normal resting permeability.

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[1] Compare my former paper, *American Journal of Physiology*, 1908, XXII., pp. 81–83. Vernon, *Journal of Physiology*, 1899, XXIV., p. 239, and Meigs, *Amer. Journ. Physiol.*, 1909, XXIV., p. 178, also regard heat-rigor as having no direct connection with the heat coagulation of the proteins of muscle.

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[1] Cf. Sachs' "Lectures on the Physiology of Plants," 1882. English translation by Marshall Ward, Oxford, 1887, p. 653.

[2] *American Journal of Physiology*, 1908, XXI., p. 200; XXII., p. 75; 1909, XXIV., p. 14.

Other instances of functional increase in permeability are apparently seen in gland cells during periods of activity. Certain influences that stimulate or heighten the irritability of muscle cells, as the action of pure solutions of many sodium salts, also, according to Fischer's and J. B. MacCallum's researches in Jacques Loeb's laboratory,[1] increase the permeability of the kidney tubules and of the intestinal epithelium; the effect is checked or counteracted by the presence of calcium salts, as also in the analogous case of muscular twitchings; and according to MacCallum hæmolytic substances — substances that increase the permeability of blood corpuscles — very generally exhibit a diuretic action, *i. e.*, increase the permeability of the kidney cells. The fact that stimulation of a gland through its nerve, as well as of a muscle, has as one of its consequences a characteristic electrical variation also indicates an increase in ionic permeability during stimulation. On the other hand, the recent results of Asher[2] with the salivary gland and the liver do not altogether bear out MacCallum's interpretation, but indicate that with such cells the specific selective power or "physiological permeability" is largely independent of externally induced changes in physical permeability. The selective action of gland cells appears indeed to be their distinctive property, and this must depend on work performed by the gland cell. Nevertheless alterations in physical permeability in all probability play an important rôle in glandular activity, as the conditions in the kidney indicate, although in the case of glands with highly developed selective properties, as the mammary gland or the liver, this factor may appear of subordinate importance.

In a rhythmically automatic tissue like cardiac muscle the phenomena of the action-current indicate on the above theory a regular alternation of periods of increased and decreased permeability corresponding respectively to the contraction and the relaxation phases of the beat. Similar rhythmical changes in permeability must be assumed to exist in other tissues that show a markedly periodic activity, *e. g.*, automatic nerve centers like the respiratory center, or contractile structures like cilia and contractile vacuoles. The case of cell-division, as seen for example in the cleavage of an egg-cell, is another instance of a rhythmical process which, reasoning from the above considerations, one might expect to find associated with, and perhaps conditioned by, a periodically recurring increase of permeability. It is in fact noteworthy that conditions which produce increased permeability in other cells — as shown by their stimulating or hæmolytic action — very generally initiate cell-division in unfertilized eggs; such are the action of hypertonic solutions, of specific chemical substances (weak acids, potassium salts, alkalis, various coagulative, cytolytic and lipolytic substances), of mechanical treatment, of temperature changes, and of the electric current under certain conditions.[1] Loeb, in a remarkable series of experiments during the past two years, has shown that, in addition to the class of lipoid solvents, such substances as saponin, solanin, bile-salts, soaps, foreign blood sera — in general substances which, as shown by their hæmolytic action, increase the permeability of the surface layers of cells — may initiate cell-division in unfertilized ova. The surface-layer of the egg first undergoes alteration with the separation of a thin film — probably a haptogen membrane consisting mainly of protein material — the fertilization-membrane. Any condition that produces this characteristic change may lead to cell-division. The process by which the membrane is formed is regarded by Loeb as of the same nature as the cytolytic process which follows more prolonged action of the membrane-forming solutions.[2] Cytolysis, however, may be simply a consequence of loss of osmotic equilibrium, as above seen. The significance of the fertilization-membrane has been the subject of much discussion. The mere separation of a thin film from the egg-surface is probably an accessory or accidental feature of the essential change involved; it seems clear, however, that one important effect must result from the removal of such a layer of material from the plasma-membrane ; the latter is, temporarily at least, *thinned* and its permeability will therefore be increased. This increase in permeability is, on the present view, the primary event in the initiation of cell-division.

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[1] University of California Publications, Physiology, 1904–5, Vols. I., II.

[2] Asher, *loc. cit.*

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[1] Cf. the recent experiments of Delage, *Archives de zoologie expérimentale et générale*, Sér. 4, T. 9, 1908, notes et revue, p. xxx.

[2] Cf. J. Loeb, *Biochemische Zeitschrift*, 15, 1909, p. 269.

Why should such increase in permeability — granting that this is the primary change induced by the above various forms of treatment — initiate so apparently complex a process as mitotic cell-division ? The main factors in producing this effect are in my opinion two : first, a disturbance of chemical equilibrium due to an increase in the rate at which certain metabolic products (probably chiefly carbon dioxide) are lost from the cell ; this is an effect similar to that which, on the general theory of stimulation outlined above, underlies the chemical effect of stimulation in muscle ; the precise effect of such a change will of course vary from cell to cell. Second, a definitely localized increase in the general surface-tension of the cell in consequence of a loss or lowering of the electrical surface polarization.

If the entire cell be regarded as a drop of fluid with a distinct tension at its boundary surface, it is apparent that a relative increase in surface-tension over the general surface of the two hemispheres combined with a relative decrease at the equator would result in a drawing of the material toward the two former areas and away from the equatorial region : a division of the drop into two might thus result. Robertson has recently drawn attention to this possibility, and by a simple but ingenious experiment has shown that a floating oil droplet may be made to divide symmetrically into two by locally lowering the surface-tension along its equator by means of a thread containing alkali or soap solution.[1]

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[1] Robertson, *Archiv für Entwicklungsmechanik*, 27, 1909, p. 29.

It would seem that the effect of locally increasing the surface-tension would vary with the mobility or fluidity of the substance composing the system. If the surface layer possessed a high degree of viscosity approaching solidity an increase in surface-tension over an equatorial band-like area encircling the cell might produce constriction — just as would occur, *e. g.*, in an inflated rubber ball if the tension were sufficiently increased about its equator. This was Bütschli's supposition, which was later favored by Erlanger and Conklin ; and by myself in an early paper. Dividing cells, however, act very plainly like fluid systems (at least in many cases, *e. g.*, starfish eggs), and there are many theoretical grounds for preferring the above point of view (see below). If the egg behaves like a drop of fluid the distribution of surface-tension assumed above would produce the observed change of form.

It can be shown by an application of the Lippmann-Helmholtz theory, connecting variations of surface-tension with changes in the potential difference at the boundary surface between two phases,[1] that increase in the ionic permeability of the plasma-membrane must result in an increase in the surface-tension of the cell. The original theory of Helmholtz[2] — that the tension between (*e. g.*) mercury and the adjoining solution is maximal with zero potential difference, and diminishes as the potential difference between the phases increases — in consequence of the static charge effects at the boundary — has required modification of recent years, since the specific effect of the ions at the boundary appears, apart from the potential difference, to modify the surface-tension.[3] Gouy's[4] extensive studies have shown that various substances, including many non-electrolytes, may modify the surface-tension of mercury. The observed surface-tension in any special case is thus dependent on (1) the specific nature of the adjoining surfaces; (2) the presence of foreign substances at the boundary, and (3) the potential difference across the boundary. In view of the sharply defined discontinuity between (*e. g.*) an egg-cell and its medium the two may be regarded as separate phases,[5] and the tension of the cell surface may be justifiably regarded as subject to the same laws as that of the mercury surface. The nature of the substances at the boundary surface and the potential difference across this surface are thus the factors to be considered. Although both are imperfectly known, certain facts seem well established. The surface film of cells contains fatty substances and proteins, both of which have marked effects in lowering the surface-tension of water; they will thus, in accordance with Gibbs' principle, tend to collect at the boundary, and the characteristics of the plasma-membrane are no doubt largely due to this tendency. The presence of such substances implies that the surface-tension of cells is at least much lower than that of pure water in contact with air; it will be still further lowered in consequence of the electrical surface polarization. The surface-tension of protoplasm is thus undoubtedly very low, and apparently under circumstances may become even negative (*e. g.*, formation of pseudopodia); the low value of this tension is shown by the readiness with which minute portions of protoplasm — amœbæ, egg-cells, leucocytes, etc. — despite the large ratio of surface to volume, undergo flowing and spreading movements. Electrical stimulation produces rounding of amœboid cells — a result to be expected if the stimulus has a depolarizing effect with consequent increase of tension; cold produces a similar change of form — a consequence, possibly, of the characteristic negative temperature-coefficient of surface-tension.

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[1] Lippmann, "Relations entre les phénomènes electriques et capillaires," *Annales de chimie et de physique*, 5me Série, T. 5, 1875, p. 494.

[2] Helmholtz, "Gesammelte Abhandlungen," Bd. I., 1882, p. 925.

[3] Cf. S. W. J. Smith, *Philosophical Transactions*, Series A, 1900, 193, p. 47. Also the papers of Warburg, G. Meyer, and Paschen.

[4] Gouy, *Annales de chimie et de physique* (7), vol. 29, p. 145; (8), vol. 8, p. 291, and vol. 9, p. 75.

[5] The criterion for regarding two contiguous systems as distinct phases is essentially that they shall be bounded by a definite surface of discontinuity exhibiting characteristic energy relations (surface tension, etc.).

A part of the low surface-tension of cells is to be ascribed to the existence of an electrical surface polarization. The evidence that this condition characterizes all living cells, while largely indirect in the nature of the case, must on the whole be regarded as strong; wherever a directly electrical test is practicable a potential difference between exterior and interior of living cells may be shown to exist, with outer surface positive; on stimulation, death or injury this potential difference undergoes a decrease.

The explanation why increase of ionic permeability produces partial or complete depolarization or fall in the potential difference between exterior and interior of the cell is briefly as follows. It is assumed that the plasma-membrane represents a partition freely permeable to the cations (probably hydrogen ions) of certain intracellular electrolytes (chiefly carbonic acid), but impermeable or difficultly permeable to the anions (assumed to be mainly HCO_3^- and $CO_3^=$) and to the undissociated molecules. This means that the velocity of the cation is practically unmodified by the membrane while that of the anion is greatly reduced. Under these conditions there will be a marked difference in the concentrations of the electrolyte on opposite sides of the membrane; the potential difference between the two solutions will be that observed between unequally concentrated adjoining solutions of an electrolyte with ions of unequal velocities. This potential difference E

according to Nernst's formula[1] may be estimated from the following values:

$$E = \frac{u - v}{u + v} \frac{RT}{q} ln \frac{c_2}{c_1}.$$

Generally, therefore, the greater the difference between the two ionic velocities the greater the potential difference across the boundary; hence this will be maximal with complete impermeability to the anion, since then its velocity is reduced to zero at the membrane and $u - v/u + v$ becomes unity. Normally, however, there is probably a certain permeability to the anion, since carbonic acid is continually evolved from living cells, although slowly in a state of rest; the potential difference in resting cells may thus be supposed to approach but not to reach the maximum. If then the permeability undergoes an increase so that anions may penetrate the membrane with considerable freeness, *i. e.*, with increased velocity, the value of $u - v/u + v$ and also of c_2/c_1 will decrease; the result will be a fall of the potential difference across the surface. The extent of this fall will obviously depend on the degree of the increase in permeability and may show a considerable range of variability.

With decrease in the potential difference the surface-tension must undergo a corresponding increase; this increase in surface-tension is, on the present theory, the main condition of the form change in cell-division. Other changes of form may naturally be similarly conditioned; irregular form changes are frequent in unfertilized eggs in which division is induced by the artificial parthogenetic methods; regular cleavage would require a symmetrical distribution of the areas of increased permeability with reference to the future cleavage plane.

In agreement with the above view of the nature of the chemical effects of stimulation, the initiation of the characteristic series of chemical processes in cell-division is to be referred to a disturbance of chemical equilibrium in consequence of the more rapid loss of certain materials, perhaps simply carbon dioxide, through the now permeable plasma-membrane. According to Lyon's[1] observations the carbon dioxide production by the dividing egg exhibits a rhythmical increase and decrease running parallel with the rhythm of cell-division; the periods of increased carbon dioxide production probably correspond to periods of increased permeability.

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[1] E is potential difference in volts, u velocity of cation, v that of anion, R gas constant, T absolute temperature, q quantity of electricity (coulombs) carried by a monovalent gram-ion, ln natural logarithm, c_1 and c_2 concentrations of the dissociated part of the electrolyte on opposite sides of the boundary surface. The ions are assumed to be monovalent in this form of the formula.

Such changes ought, on the membrane theory, to be accompanied by changes in the state of electrical surface polarization. Experimental proof of the existence of such changes is difficult but probably not altogether impracticable. Miss Hyde has attempted — using the capillary electrometer, with one electrode on the blastodisc of dividing fundulus eggs, the other on the opposite pole, — to determine if, during cell division, any potential difference makes its appearance between the dividing portion of the egg and its unaltered general surface.[2] While her results are not altogether unequivocal, they do seem to indicate that at or about the time of cleavage the outer surface of the blastodisc becomes negative relatively to the general outer surface of the egg — *i. e.*, undergoes a change of potential similar to that which occurs during stimulation, indicating increased permeability. These important and interesting experiments should be repeated and confirmed. There are in fact various indications that during cell-division the potential difference between the exterior and interior of the cell undergoes marked alteration. The disposition of the colloidal material of the cell at this time in the characteristic radiations is strong confirmatory evidence; this phenomenon plainly suggests the polarization of colloidal particles in a strong electrical field. It is a corollary of the above theory that with the appearance of an increased permeability — implying depolarization of the surface layer — the peripheral regions of the protoplasm must become — for a time at least, until the potentials are equalized — *positive* relatively to the interior. If the voltage of the surface potential change is comparable to that of the action-current of muscle, *i. e.*, *ca.* 0.05–0.08 volt (as is highly probable), a potential gradient of considerable steepness will exist temporarily, at the time of increased permeability, between the peripheral and the central regions of the protoplasm; this may be amply sufficient to account for the striking change in the configuration of the cytoplasmic colloids.[1] The peripheral layer would become positive relatively to the central region under the conditions existing in such a system as a cell with a surface polarization of the kind indicated. If we can draw safe inferences from the cytological facts the astral centers do in fact show the properties of negative regions.[2] I hope however to consider the physiology of cell-division from the above point of view more fully in a later paper.[3]

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[1] E. P. Lyon, *American Journal of Physiology*, 1904, XI., p. 52.

[2] I. H. Hyde, *ibid.*, 1904, XII., p. 241.

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[1] It may be desirable to give here the estimate on which this statement is based. If we assume that the surface potential difference due to the physiological polarization has the same value as the potential difference of the demarcation current of muscle (*ca.* 0.08 volt) and that the fall of potential or negative variation during cleavage is equal to that of the action current (*ca.* 0.05 volt), we infer that in the resting cell there is a potential difference between exterior and interior equal to *ca.* 0.08 volt. This gradient, being due mainly to the unequal permeability of the plasma-membrane to the two ions, exists almost entirely between the inner and outer surfaces of the plasma-membrane; the interior of the cell (cytoplasm) would thus have an almost uniform negative potential of about this value with little if any fall between its central and peripheral regions. If now, in consequence of a sudden increase in permeability, the potential difference across the plasma-membrane decreases by 0.05 volt, a gradient will temporarily exist between peripheral and central regions of the protoplasm equal to this value, the most negative region being that most remote from the periphery. A gradient of 0.05 volt between the center and the periphery of a sea-urchin egg, diameter 0.072 mm., is a gradient of 0.05 volt in 0.036 mm., *i. e.*, 1 volt in 0.072 cm. or about 14 volts per centimeter. The assumed potential differences may be too high, though they are typical of the directly observed values in muscle cells. The change in permeability is also probably rather gradual than sudden. At least these considerations show that a strong electrical field may arise between central and peripheral regions of the cell under the conditions assumed. Of course no such potential difference could be *maintained* without the performance of electrical work on the part of the cell (assuming the free movements of ions within the cytoplasm); but its existence for only a few seconds might well produce the observed effects. This note, however, aims merely at justifying the above statement in the text, not at a complete discussion.

[2] Cf. my paper in *American Journal of Physiology*, 1905, XV., p. 46.

[3] In my first paper dealing with the theory of cell-division (BIOLOGICAL BULLETIN, 4, 1903, p. 175) the system of astral radiations was recognized as evidence of an electrical potential difference between peripheral and central regions of the dividing cell, and the form change was ascribed to a capillary electric change of surface-tension. The tentative hypothesis then presented to account for the general course of the process was, however, quite different from that outlined above, and, with the exception of certain details, will have to be abandoned.

A REËXAMINATION OF THE CYTOLOGY OF HYDRACTINIA AND PENNARIA.[1]

W. M. SMALLWOOD.

The present report does not claim to be a complete discussion of the cytological phenomena in the developmental history of *Hydractinia* and *Pennaria*, nevertheless, it adds considerable new data hitherto unpublished concerning the problem of the structure of the egg, the migration of the chromatin, maturation, fertilization and mitosis. However, the present paper cannot claim to settle the controversy as to the existence of amitosis because the results concerning which there may be some differences of opinion have been negatively interpreted.

During the summer of 1906 Professor C. W. Hargitt asked me to undertake a reëxamination of some of his work on hydroids paying particular attention to the cytological phenomena of the early development, that being the point of most interest. The results of Bunting, '94, on *Hydractinia* were not in agreement with his observations on *Pennaria*, *Eudendrium*, *Clava*, etc., so that he suggested that it might be well to restudy this form also. While I have had opportunity to examine all of Professor Hargitt's preparations on the several hydroids studied by him, it seems wise to confine this paper to *Hydractinia* and *Pennaria*.

It is not often that such a study as this is undertaken and when it is there is involved a great deal of work that it would be superfluous to republish so that the present paper needs to be read in connection with Bunting, '94, on *Hydractinia* and Hargitt, '04, on *Pennaria*. At the beginning of this study, Professor Hargitt explicitly stated that he wished to give me absolute freedom in the problem and the interpretation of the results. This he has done even to the extent of not seeing any of my preparations until I turned over the finished paper to him.

When he asked me to undertake this restudy, he volunteered to collect and preserve the material necessary. In this particular too much credit cannot be given Professor Hargitt for his persistent experiments in trying to find a suitable fixing reagent for these refractory eggs of hydroids. Had I been without the benefit of his long experience, I doubt if the present results could have been secured. He used, among fixing agents, Bouin's fluid which has given the best fixation of any thus far tried and certainly these preparations are superior in regard to their fixation to any which he made during the preceding years of study. There is very little doubt that the eggs of hydroids degenerate when left in alcohol for some time and should be embedded in paraffin as soon as possible.

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[1] Contributions from the Zoölogical Laboratory, Syracuse University, C. W. Hargitt, director.

It seems unnecessary to review the general literature on this subject for this has already been well done by Hargitt in his numerous papers and by Bigelow, '08. Professor Hargitt is so well known as an authority on hydroids that it has seemed unnecessary for me to make an elaborate critique of his papers. Therefore, I have in most instances simply referred to the pages where he discussed similar phenomena, elaborating only those points upon which I have additional data.

Origin of the Eggs in *Hydractinia*.

The eggs arise in the entoderm close to the basement membrane. Certain entoderm cells are directly transformed without any immediately preceding cell division. These young ova are distinguished by having a large nucleus and granular cytoplasm. No special region in the polyp is devoted to the production of ova as Bunting, '94, maintains and text-figures 1 and 2 demonstrate. The young ova are as likely to begin their growth in the cells in the base of the polyp as in those along the side.

Growth of the Egg in *Hydractinia*.

The first apparent change in these entoderm cells which are to become ova that has thus far been observed is a marked increase in the size of the nucleus before the cell as a whole has undergone any change. The result is that the nucleus occupies most of the cell, the cytoplasm being limited to a narrow border. A comparison of the nuclear contents at this early stage with the surrounding entoderm cells shows that the nucleolus is increasing rapidly in size. In the entoderm cells it is so very small that it is difficult to be sure of its presence in some cells, and the same is true in some of the young ova, but in most instances it is a clearly defined and easily distinguished body.

The most interesting change is found in the chromatin network. In the young ova it is now in its most conspicuous state. From this time on until new ova arise in a new polyp the chromatin does not possess such distinctness. A loose network is distributed through the nucleus with conspicuous masses where the threads cross. The achromatic substance (residual substance of Lillie, '06) does not stain in the acid or basic stains. The nuclear membrane is very distinct, chiefly due to the fact that a considerable amount of chromatin material seems to be directly in contact with it. The cytoplasm is as yet free from the microsomes — minute granules — which characterize the slightly older ova.

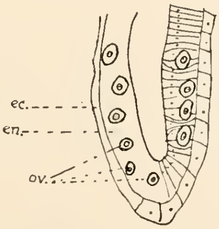

FIG. 1. Outline drawing of base of polyp to show the position of the ova. *ec*, ectoderm; *en*, entoderm; *ov*, ova. *Hydractinia*.

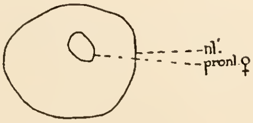

FIG. 3. Camera lucida drawing of the egg nucleus, the larger circle, and the female pronucleus, the smaller circle. *Nl*, nucleus; pronl. ♀, female pronucleus.

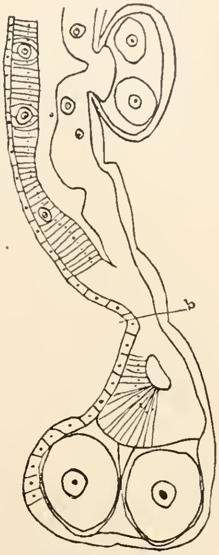

FIG. 2. Outline drawing to show that sometimes the Hydractinian polyp branches. *B*, base of polyp. Reconstructed from several sections.

The next step in this gradual transformation is the rapid increase in the cytoplasm, accompanied by a growth in the nucleus. The cytoplasm is now loosely sprinkled with microsomes, in places giving the appearance of a reticulum. With the accumulation of the microsomes, the reticulate condition is obliterated and the cytoplasm becomes a dense mass of microsomes. In such young ova the achromatic substance of the nucleus takes a plasma stain such as Orange G or Bordeaux red. The vacuolation of the nucleolus has begun by this time, the vacuoles taking the plasma stain. This vacuolation of the nucleolus is in agreement with the many cases already described. The vacuolation continues until the nucleolus disappears which occurs before maturation. The growth, vacuolation and eventual disappearance of the nucleolus is an event which takes place during the growth of the egg, but is not synchronous with any definite phase of this growth.

The young ova thus described are found in the entoderm at the base, the side of the polyp, or in the gonophore. About this time the ova take their permanent position (Bunting) in the gonophore, *i. e.*, in the ectoderm where they increase greatly in size. This increase in size is largely a matter associated with the growth of the individual microsomes into small spherules. We are led to believe from this study that there are also many microsomes added which likewise are changed into spherules. These spherules are so numerous in the adult egg as to conceal the ultimate structure of the cytoplasm. They take a dense stain but not always a homogeneous one. During this gradual growth, the staining reaction changes, a change which can be readily seen on a slide where all stages are represented. A number of such were studied where all of the material had received the same treatment from fixation to and including staining. On such a slide the young ova are so deeply stained as to conceal most of the details of structure while the large eggs show but a faint response to the stain. But when the more mature eggs are once stained in iron hæmatoxylin, it takes considerable time to differentiate them in the iron so tenaciously do they hold the stain. Under these conditions one may see on the same slide young ova black and blue-black in color while the older eggs are hardly stained at all. Between these two extremes, there are all gradations. This change in color reaction is evidently due to the transformation of the microsomes into spherules.

Chromatin Changes in the Egg of *Hydractinia.*

While the cytoplasm is becoming thus transformed, the nucleus has increased in size although it does not become more distinct. The nucleolus is mostly occupied by a large vacuole. In place of the sharply defined chromatin masses in the younger ova, there is a marked change in this particular. The typical chromatin reaction is hardly evident and the whole nucleus tends to take a stain similar to the cytoplasm. The chromatin threads and masses are less definite in position and arrangement. The question naturally arises, what is becoming of the chromatin? Can its disappearance be traced into any part of the egg? In other words, is there a definite and specific migration of chromatin from the nucleus into the cytoplasm? The following facts are submitted in answer to these queries.

While the eggs are still in the gonophore and the nucleolus is becoming vacuolated, small particles of chromatin leave the nucleus and wander out into the cytoplasm. Fig. 1, Pl. I., shows the early stages of this process, some of the chromatin granules are just emerging. Others have moved some distance. My attention was first directed to this phenomenon by finding eggs which showed conditions such as Fig. 2, Pl. I., typifies. A number of densely staining chromatin granules are scattered in the cytoplasm and mostly surrounded by a narrow clear area. These masses of chromatin are small and usually single but occasionally two or three are found in a single vacuole. The reason for regarding these masses as chromatin is because they give the same color reaction as similar shaped bodies in the nucleus; and for the further reason which is obvious in Fig. 1, namely, the actual migration of the chromatin from the nucleus.

When the size of the nucleus of the mature egg, *i. e.*, before maturation, is compared with the female pro-nucleus one is strongly impressed with the great reduction in size. Text-fig. 3 is a camera lucida drawing of the outline of the egg nucleus as represented by the larger circle, while the smaller circle within represents the size of the female pro-nucleus. It must be apparent at once that up to and during maturation there is a remarkable reduction in the size of the nucleus which the maturation phenomenon alone does not adequately explain. The nucleus loses a large amount of chromatin by direct migration into the cytoplasm which is entirely independent of the chromatin discharged during the formation of the polar bodies. The subsequent fate of this discharged chromatin has been studied with much pains and it is the belief of the writer that it is something as follows: When the cytoplasm of the egg is examined after it has been discharged from the gonophore there appear many areas that are free from the characteristic granules of the surrounding cytoplasm. These areas are usually round and contain small particles that stain with Borax carmine or hæmatoxylin. They look so much like faintly staining nuclei that their appearance is very confusing at first (Fig. 6). As segmentation progresses, these areas tend to migrate to the periphery of the egg and are occasionally so numerous that they form a nearly continuous row around the embryo, Fig. 9. Eventually they are absorbed by the cytoplasm. This explanation, then, traces the highly vacuolated condition of the cytoplasm in *Hydractinia* directly to the migration of chromatin from the nucleus before maturation begins. A similar series of changes occurs in *Pennaria* but at a different time.

Localization of the Formative Stuffs in *Hydractinia*.

The following extract indicates the extent of the previous description of the structure of the cytoplasm. "Sections of the egg show deutoplasm spheres distributed throughout the protoplasm, with the exception of the outer rim which is composed entirely of protoplasm" (Bunting, page 215).

The cytoplasm exhibits a rather definite localization of the so-called formative stuffs in the presence of a coarsely granular crescentic area (picro-acetic fixation) located on the side of the egg in which the nucleus lies — the animal pole. The appearance of these granules in *Hydractinia* is very similar to what Hargitt, '06, p. 214, has found in *Clava*. In addition to these bodies there are some minute bodies located around the periphery of the egg in a narrow band which takes a deep blue stain (Borax carmine, Lyons blue method). These particles do not stain readily and so were overlooked by Bunting. By the regular hæmotoxylin methods they are usually indistinguishable from the other microsomes and spherules. This gives three distinct bodies in the cytoplasm: (1) The ordinary bodies all through the cytoplasm and usually interpreted as yolk masses; (2) the coarse granules distributed in crescentic bands; (3) the small bodies around the periphery. The small peripheral granules remain on the outside of the embryo during cleavage and can be traced into the planula. In the planula they are confined to the ectoderm. The differentiation and subsequent fate of similar particles has been made out in *Pennaria*. The first and second class of granules are chiefly confined to the mass of cells within the ectoderm of the planula, although a few scattering granules are seen in the ectoderm.

MATURATION IN *Hydractinia*.

The following quotation reveals the extent of previous observations on this phase of development. "The ovum while in the gonophore has a well-defined nucleus situated above the center of the egg, which fades from view when the ovum is deposited" (Bunting, page 215). "In about fifteen minutes after the ova are laid the polar bodies appear. When first observed two globules were present, one had been extruded, while the other one was just appearing. One of the two divided subsequently, in a plane at right angles to the first cleavage plane of the ovum. Within ten minutes from the extrusion of the first polar body, the second was ejected" (Bunting, page 216).

The nucleus during growth varies from round to elliptical and in this latter shape the ends may nearly reach to the periphery but until maturation begins, the nucleus is central in position. It is to be regretted that more stages in maturation have not been discovered notwithstanding the fact that large numbers of slides have been made of eggs just after deposition. Maturation begins before the eggs are discharged from the gonophore but just how long I am unable to state. The fact that this process begins while the eggs are still retained, makes the solving of the problem tedious in as much as the gonophores are not set free as are the medusæ in *Pennaria*. Occasionally a gonophore containing eggs is found among the recently discharged eggs and it was in such that the first signs of maturation were detected. A large number of slides were made of the large gonophores from colonies that were laying eggs when fixed but none of these showed any of the maturation phenomena. Fig. 3, Pl. I., shows the prophase of the first maturation. The asters have moved part way around the nucleus and a few spindle fibers are evident. The nuclear wall on the side toward the middle has been partly broken down. The chromatin shows but a slight tendency to take a stain although it is collecting into definite masses. The chief importance of this drawing centers around the process by means of which maturation occurs, namely, the mitotic process.

When this material was collected it was not thought that maturation began until after deposition because of the observations quoted. For that reason much time was spent studying the eggs just after deposition, but in all cases none of the earlier stages were found. In two or three eggs undoubted polar bodies were found after the eggs had been deposited, and such a case is shown in Fig. 4, Pl. I. Fig. 4 represents the late telophase of what I judge to be the second maturation. In the polar cell there are several vesicles which probably represent chromatin. In the egg the chromatin vessels are very small and grouped among the remains of the breaking down astral fibers. These vesicles collect into a single vesicle, the female pro-nucleus, Fig. 5, Pl. I.

Certainly these two figures do not furnish a complete account of maturation, but they do show: (1) The nature of the process; (2) that this process begins in the gonophore; (3) that Miss Bunting's observations are not correct, because if what she observed were polar bodies they would be found attached to the egg before and after segmentation, but such is not the case. The polar bodies usually drop off as soon as the eggs are discharged. In all of the eggs studied but two were found which retained the egg nucleus after the egg had been deposited.

One of the curious conditions is shown in Fig. 5, where the female pro-nucleus lies next to the periphery of the egg with a few fibers apparently starting from its outer pole. This was thought for some time to mean the prophase in maturation, but never could fibers or an aster be found at the inner pole. Just why there should be a small furrow over this nucleus I do not know, and such a furrow does not always occur. It might be thought to mean the beginning of segmentation, but such a furrow may appear before fertilization takes place. In a number of instances the nucleus in this same stage was seen to be partly protruding from the substance of the cytoplasm, a condition for which no explanation is offered. The chromatin in the female pro-nucleus takes a faint stain up to the time of the first cleavage. The differentiation of the egg of *Hydractinia* is very difficult, much more so than in *Pennaria*, which makes the recognition of these very arduous. In the region of the nucleus in Fig. 5 there are several deeply staining particles which look much like chromatin, but of their nature I am uncertain. That this nucleus is the female pro-nucleus the following reasons indicate: (1) The absence of the nucleolus; (2) its relatively small size; (3) that it has been traced directly into the first cleavage.

FERTILIZATION IN *Hydractinia*.

Thus far no sperms have been found in the eggs before they were deposited but after deposition spermatozoa are seen in contact with the eggs. The sperm head becomes transformed into a vesicle soon after it penetrates the cytoplasm. There does not seem to be any definite place where the sperm enters the egg. During the progress of the male pro-nucleus through the cytoplasm, no aster was seen nor any definite path. The staining reaction of this body is so very faint that it is made out only after careful study with the oil immersion.

Fig. 6, Pl. I., shows the approach of the male pro-nucleus and the change in shape of the female pro-nucleus preparatory to the prophase of the first segmentation. No asters or radiations could be distinguished in connection with either of these pro-nuclei at this stage. These observations further show that the egg nucleus does not "fade from view when the ovum is deposited" but that it can be traced continuously from the egg in the gonophore to the first segmentation stage.

CLEAVAGE IN *Hydractinia*.

The difficulties encountered in differentiating these eggs has made it almost impossible to discover a complete series of the changes in any of the stages as many of the mitotic phenomena are visible only with the oil immersion lens. This means that but few eggs will be cut in just the right plane to enable one to make out the correct relations ; and it also means that important conditions will escape detection.

The first division of the egg into the two-cell stage is preceded by the formation of a definite mitotic figure after the male and female pro-nuclei have come together. The chromatin becomes more responsive to stain and gradually condenses into a definite number of very minute chromosomes. These chromosomes in size and staining reaction are so similar to some of the granules in the cytoplasm, Fig. 8, Pl. II., that it is impossible to be certain that they are chromosomes unless the spindle fibers are present. This makes the determination of the number of chromosomes difficult because when one has a cross-section of the metaphase through the equatorial region, one cannot determine the relation of the spindle fibers to the chromosomes and so cannot be certain of their number. The task was a little easier in *Pennaria*, where fourteen were counted in the anaphase, although here I am not certain that this is the correct number. I think that there are from twelve to sixteen chromosomes present in these hydroids, the exact number remains to be determined.

The chromosomes form in the typical metaphase condition, split and move toward each pole of the spindle. In the anaphase distinct interzonal fibers are present. During the telophase the chromosomes are transformed into a nucleus. This nucleus does not necessarily assume the rounded outline but is often elongated and even irregular in shape. The prophase of the next cleavage frequently begins while the nucleus is in this condition, Fig. 7, Pl. I. In Fig. 7 a typical prophase of mitosis in cleavage is shown. The faint asters are on opposite sides of the elongate nucleus, and a few spindle fibers are forming preparatory to the metaphase and the dissolution of the nucleus. The centrosphere as shown in Figs. 7 and 8 will be discussed in a separate section.

A definite mitotic figure has been made out in all of the early stages of segmentation and followed up to the planula stage. A typical condition of the early embryo is shown in outline in Fig. 9, Pl. II. The cells surround a cavity which is first seen in the two-cell stage and is due to the separation of these first two cells.

This cavity increases in extent as segmentation continues. After a time the peripheral cells begin to segment in such a manner as to set cells free in this cavity. The direction of the spindle in Fig. 8 shows the method. Fig. 8 was taken from one of the cells shown in Fig. 9 and in each of the remaining blastomeres there is a mitotic figure, so placed as to give rise to a cell that becomes free in the segmentation cavity. The segmentation cavity finally becomes full of cells due to the setting free of cells from the periphery and the subsequent division of these same cells as they lie in this cavity.

MEMBRANES IN *Pennaria*.

First or False Membrane.—The absence of a membrane or membrane-like structure in the animal egg is doubted by some notwithstanding that Wilson, '82, Metschnikoff, '86, Hargitt, '04, and others have repeatedly stated that the eggs of hydroids are naked. Brauer, '91, finds in *Hydra*, after the embryo is formed, two membranes produced by the ectoderm, but the unsegmented egg is naked. Previous studies in *Pennaria* make no mention of an egg membrane, but the ectosarcal portion of the egg is described by Hargitt as forming at times a filamentous membrane around the cytoplasmic papillæ. Aside from these references no mention is made of membrane in hydroids.

When the eggs of *Pennaria* are well fixed in Bouin, and a hæmatoxylin stain is followed by a plasma stain such as Bordeaux red, a rather broad but uneven structure is readily observed. It first forms while the egg is still growing and is easily made out while the eggs are still in the medusa. The portion of the egg adjacent to the manubrium shows pseudopodia-like processes (cf. Hargitt) and between these processes this structure is quite wide and irregular in width. After the eggs are deposited and have assumed the rounded outline, this membrane-like structure appears as shown in text-fig. 4. It is rarely of uniform thickness and usually shows one place that is bulging. Also

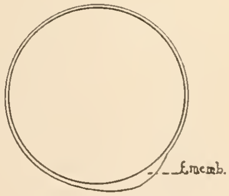

FIG. 4. Outline drawing to show the relative thickness and variability in width of the false membrane. *F. memb*, false membrane.

in the laid eggs as segmentation begins and continues, it appears torn so that some of the eggs will be only partly surrounded by it. Some time during the progress of cleavage this membrane disappears entirely. It is well known that the newly deposited eggs of *Pennaria* are inclined to stick to bits of grass, a glass dish, etc., which is due to the adhesive property of the false membrane. The term false membrane is applied to this structure because it is not permanent; and the term membrane is used because it seems to serve the purpose and do the work of a real membrane. No differential lamellæ were visible in this false membrane either before or after deposition. Numerous sperm heads are frequently to be distinguished within its substance. The inequalities in thickness of the false membrane suggest that this substance is of a fluid nature in the living egg and transparent. If this interpretation prevails, then one can no longer speak of the eggs in *Pennaria* as being naked.

Second or True Membrane. — After fertilization and with the beginning of cleavage a second membrane begins to form (cf. Hargitt) which lies in close contact with the granules of the cytoplasm and completely surrounds each cell as it is produced in cleavage. This structure compares favorably in appearance, staining reaction, etc., with the regularly described egg membranes of animal eggs and so is designated as the true membrane.

Maturation of the Egg in *Pennaria*.

From the early development of the egg up to the beginning of maturation I cannot add any new data, but do confirm Hargitt's ('04, p. 456) observations. The extensive observations of Hargitt as well as my present independent studies indicate that it is very rare to find a polar body attached to the egg after deposition, not one in one hundred will show a polar body at this time. It is also very rare to find a deposited egg that still retains the egg nucleus with a nucleolus. In fact this latter structure, the nucleolus, has been taken as an indication of whether maturation has taken place or not. Where it is lacking I have designated the nucleus as the female pro-nucleus. The egg nucleus in *Pennaria* does not show any such great size as is found in *Hydractinia* and varies but slightly from the female pro-nucleus which is always present in the egg previous to the beginning of segmentation.

Sections of medusæ after they have become free from the colony and before the eggs are discharged may show the presence of polar bodies. Several different slides show conditions such as are indicated in Fig. 10, Pl. II. The first polar body is being shoved to one side by the formation of the second. The chromatin is in the form of three vesicles in the first polar body. Even while the egg is in the medusa, the polar body may be pushed some distance from the spot where it emerged which may be due to the contractions of the bell of the medusa. The second polar body has ten vesicles in two groups ; while thirteen are made out deeper in the egg. To the right there is one isolated vesicle. This one and several of the thirteen contain granules of chromatin. This interpretation of the vesicles grows out of my study of the changes through which the chromatin passes during cleavage. The several vesicles deep in the egg unite into a single definite nucleus like the one in Fig. 11, Pl. II. That these polar bodies are formed by the mitotic process, there is but little doubt because of similar conditions in *Hydractinia* where there can be no doubt as to the beginning of the process ; and because of the state of the chromatin, which in this condition of several vesicles, is entirely unlike the amitotic process of division.

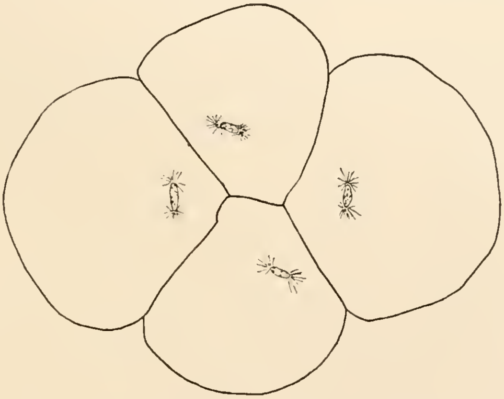

FIG. 5. A regular segmentation stage, all cells in prophase.

In the same medusa from which Fig. 10 was taken there were three other eggs in the same phase of maturation. *Pennaria* was collected by Professor Hargitt early in the morning and in the afternoon but in the numerous sections made of the adult medusæ, none showed any earlier stages. With this data at hand, however, it should be an easy matter to secure the intervening stages which undoubtedly occur about the time the medusæ are set free. Sections of medusæ still contained one or two eggs which in each instance had completed maturation, Fig. 11, and the polar bodies were not in contact with the egg. The conditions shown in Figs. 47 and 49 by Hargitt are without question polar bodies and probably the second. The state of the chromatin vesicles in Fig. 47 is more regular than any that I have seen which may be due to the preservation. They seem also to be more scattered than any observed in eggs fixed in Bouin. The clear area within the egg just beneath these vesicles was not seen in the Bouin fixation. Another puzzling feature of this study on maturation was the presence of small bodies attached to the surface of the egg. They were especially noticeable on the eggs of *Hydractinia*. After some study, it was apparent that they were protozoa which in many instances looked exactly like polar cells found in mollusca.

CHROMATIN CHANGES IN *Pennaria*.

The female pro-nucleus is a small, faintly staining body that is found in eggs that are just laid and many that are still in the medusa. Sometimes it was pointed at the outer end and pushed close against the false membrane but it was never found protruding. It occupies this position until the approach of the male pronucleus when it may move some distance from the periphery of the egg. But before the fusion of the two pro-nuclei, there occurs a series of unusual changes in the chromatin, especially of the female pro-nucleus. Changes of a similar nature but not as extensive are found taking place in the male pro-nucleus. The chromatin changes described in *Hydractinia* preceded maturation. These in *Pennaria* follow maturation.

It is difficult to determine whether there is any order to these changes and so no attempt is made to decide which is the older state in the series of Figs. 12 to 18. In several of these figures the male pro-nucleus is shown but its position, near or far from the female pro-nucleus does not seem to influence the time when the chromatin is to migrate from the nucleus. Immediately in contact with each pro-nucleus, the cytoplasm becomes denser and is composed of finer granules (cf. also Hargitt, Figs. 48, 49, 50). But isolated or wandering nuclear vesicles usually lack this modification of the cytoplasm which has been used to assist in recognizing the pro-nuclei.

Figs. 12, Pl. II., 15 and 18, Pl. III., show some of the chromatin granules close to the nuclear membrane as if they had just emerged from the nucleus into the cytoplasm. In both Figs. 15 and 18 these chromatin granules are on opposite sides of the nucleus which indicates that their position is not the result of the accident of cutting the sections. The male pro-nucleus in Fig. 15 shows the same condition of the chromatin. After the chromatin has been in the cytoplasm for some time, there are found definite small vesicles usually empty. From the study of mitosis in cleavage and the changes in the chromatin during the anaphase and telophase, the suggestion that these vesicles are the product of the transformed chromatin seems inevitable. During this period, while the chromatin is migrating into the cytoplasm, the chromatin both within and without the nucleus takes a very faint stain so that the whole nucleus is easily overlooked. Some of the most satisfactory results were obtained by using Brazilian without the iron mordant. In no instance have nuclei entirely devoid of chromatin been found. The vesicles in Figs. 13, 14, 17, Pl. II., and most of them in Fig. 12, are empty. If the interpretation offered is correct, then there must be a very large amount of chromatin that leaves the nucleus in Fig. 12. The meaning of the large flask-shaped vesicle attached to the female pro-nucleus in Fig. 15 is not understood.

There is some question as to whether this process takes place in all of the eggs preparatory to cleavage, but that it is very common and appears in well fixed eggs there can be no question. On the same slides were found mitotic figures preserved in all of their parts. The slides showing many of these phenomena were examined by Mr. George T. Hargitt who is at work on a similar problem at Harvard University and he confirmed the correctness of these observations. The conditions certainly exist as drawn but more drawings could be made easily showing different relations of the chromatin masses and vesicles. The eggs just after deposition do not show these chromatin changes. After a time the chromatin that remains in the male and female pro-nuclei increases in amount and takes a deeper stain until the chromatin appears like that shown in Figs. 16, Pl. IV., and 19, Pl. III. Hargitt in Fig. 48 has two similar vesicles.

The vesicles last but a short time and usually are indistinguishable by the time that segmentation begins. But when such conditions as shown in Fig. 16 exist in the presence of a small vesicle containing chromatin near the female pro-nucleus and a second one between the two pro-nuclei, these remain longer in the egg. What their influence on subsequent development is, some light may be gained by a study of Fig. 49 (Hargitt). In this drawing there is a clearly defined mitotic figure with faint chromosomes; and a short distance away, three asters and their connecting fibers are around several vesicles. This Fig. 49 seems to be a later stage in the transformation of some of these vesicles containing chromatin and indicates a pseudo-segmentation in that it is not preceded by the fusion of the male and female pro-nuclei. It hardly seems as if such conditions played an important part in the future segmentation.

Fertilization in the Egg of *Pennaria*.

The spermatazoa penetrates the egg of *Pennaria* frequently before deposition, but the penetration is usual after the egg is laid. The false membrane may contain a large number of sperm heads that were unable to gain admission into the egg. The sperm head immediately after penetration becomes transformed into a vesiculate body, the male pro-nucleus. No definite place of entrance, nor path through the cytoplasm, nor the presence of asters could be determined. It remains smaller than the female pro-nucleus and is surrounded by fine cytoplasmic granules. Figs. 15, Pl. IV., 16, Pl. III., and 18, Pl. IV., give a good idea of the male pro-nucleus.

Figs. 17 and 19 show the approach of the pro-nuclei. The fine cytoplasmic granules surrounding the two bodies have united in Figs. 15 and 19 and may have a stellate outline. It was practically impossible in Fig. 19 to determine whether there was a fusion at this stage of the two nuclei or whether they were merely in contact.

Polyspermia. — In Fig. 20, Pl. IV., there are shown conditions which seem to point strongly to polyspermia. The female pro-nucleus is present and some distance away a single vesicle which is similar in every respect to the male pro-nucleus of later stages. The cytoplasm is indented just opposite as if modified by the recent penetration of the sperm. Then close by this vesicle are nine other vesicles, somewhat smaller but otherwise identical with the single one. There is likewise the same modification of the cytoplasm just opposite. The natural explanation seems to be to regard these all as transformed sperm heads, and consequently polyspermia. Fig. 21, Pl. III., shows a second case of probable polyspermia where there are three nuclei, one much larger which is probably the female pro-nucleus. The other two are interpreted as male pro-nuclei. That several nuclei may exist previous to the first segmentation like this Fig. 21 and Fig. 48 of Hargitt there can be no question and that they are in some instances due to polyspermia I feel equally certain. But that this is the exclusive interpretation I do not accept because nuclear division may outrun cytoplasmic cleavage which results in giving several nuclei in the cytoplasm as Figs. 43 and 43*a* of Hargitt's clearly indicate and I have confirmed. It seems more probable that both explanations should be used in interpreting the multiple nuclear conditions.

CLEAVAGE OF THE EGG IN *Pennaria*.

This part of the paper on cleavage tends to show the manner of cleavage rather than a detailed description of the process as this has already been done by Hargitt. As was described in the section on fertilization, the two pro-nuclei approach preparatory to the first cleavage. Fig. 22, Pl. II., shows the prophase of the first cleavage. The female pro-nucleus has become much elongated and there are distinct asters at each end. The spindle fibers are just forming and the chromatin is still in the loose stage ; the chromosomes are yet to form. The male pro-nucleus, such of it as appears in the section, is near one end of the female pro-nucleus and much smaller. In Fig. 23 is shown another form of the prophase. This drawing was taken from the beginning of the second cleavage. The small prophase spindle lies entirely within the centrosphere and the chromosomes are in the form of vesicles. That these vesicles are modified chromosomes is proved by the condition of the chromosomes at the opposite pole of the old spindle where the process was not as advanced. Here some of the interzonal fibers were still in contact with the partly transformed chromosomes. The successive cleavage is so rapid in this instance that the new spindle has formed before the vesicles have united into a single vesicle, the nucleus. Nevertheless a normal spindle will result when the chromosome vesicles are transformed again into chromosomes. It is the study of such changes as these in the chromatin that convinced me that the vesicles in Fig. 10 were modified chromosomes and the vesicles in Figs. 12–16 were formed through the influence of chromatin astray in the cytoplasm. A third form of the prophase is quite common, Fig. 24, Pl. IV., where the nucleus is much elongated or somewhat irregular in outline. This prophase stage is farther advanced than the two previously described. The asters are larger and the forming spindle fibers more pronounced at each end. The chromatin has begun to take a deeper stain. In some respects all of these three forms of prophase are different, but the differences are not fundamental, and plenty of similar variations are known in other animals. They are all unmistakably by the mitotic process.

The metaphase is as typical as exists in any mitotically dividing cell. The chromosomes split and move to each pole, Figs. 23, 26, 27, Pl. II. During the late anaphase and early telophase the chromosomes become transformed into vesicles, Figs. 27, 23, may or may not unite into a single vesiculate nucleus before the next cleavage. I have been able to trace the nucleus continuously from its state in the unmaturated egg through all of the cleavage states. At no times does it dissolve other than in the normal mitotic changes. At no time does the total contents of the nucleus become dissipated throughout the cytoplasm to reform into separate nuclei. Other than the chromatin changes just previous to segmentation, the nuclear phenomena in *Pennaria* appear perfectly normal.

Sometimes the cleavage is very regular as text-fig. 5 indicates, each cell shows the nucleus in the prophase and apparently so placed as to give off cells in the clockwise directions. The tilting of these nuclei and the perfect regularity is so apparent that this drawing might have been taken from an annelid or molluscan embryo, but in this same group of cells there was a most irregular embryo so that this regular condition is nothing more than an accident and is the only regular embryo among many sections.

The Centrosphere in the Egg of *Pennaria*.

It is difficult to decide on any of the old terms to describe the conditions in *Pennaria*. If the term centrosphere may include sphere and centrosome, the latter being potentially present only, then this may be taken as an acceptable term.

If there is a centrosome present in these eggs then it is an unstable body which varies greatly and is recognized with much difficulty. In all cases of the prophase, there remains a small clear area from which the fibers radiate but into which they do not penetrate. Fig. 24, Pl. III., shows a few fine granules in this area but their size, number, and staining reaction does not indicate that they are at all constant, nor can one detect that there is any inherent relation between these granules and rays or spindle fibers. That there is probably some substance that plays the part of a centrosome is apparent in Fig. 23, Pl. III., where the whole new spindle lies entirely within the old centrosphere. Therefore, it seems as if one might say that certain of these granules have potential centrosome properties and are possibly in the way to become differentiated; that is to say, that the elaborate centrosomes of mollusca for example indicate a higher degree of differentiation, while in these hydroids a similar result is produced by several granules which cannot be differentiated from the rest of the sphere substance.

The transformation of this centrosphere keeps pace with the changes in the chromosomes. By the time that the anaphase is reached the centrosphere substance has increased greatly in size, Figs. 25, 26; and in late anaphase is very conspicuous, Fig. 27. After the metaphase stage, the astral fibers are mostly composed of granules, Figs. 25, 26. The interzonal fibers persist for some time and are frequently bent as the cleaving cytoplasm passes through them. At such times, the chromosomes lie at each end of a V-shaped figure. As the fibers disappear and the chromosomes become transformed into a nucleus, the centrosphere substance largely becomes an indistinguishable part of the cytoplasm, but a small portion remains as a clear, narrow region surrounding the nucleus. This means that the deeply staining, newly formed "resting nucleus" with a narrow transparent area around it in cleavage is perfectly normal, being the last remains of the centrosphere.

PAPILLÆ IN *Hydractinia* AND *Pennaria*.

Hargitt (p. 469) has described these as ectosarcal phenomena. My study confirms his and shows that in both of these hydroids the papillæ are common in the unsegmented egg and have even been seen while the egg was in the medusa. There is no particular region on the egg where they arise. At first these papillæ were regarded as polar bodies, especially in *Hydractinia*, but when they were found on the vegetal pole and the female pro-nucleus was present at the animal pole in the same egg, such an interpretation was impossible. I believe that these papillæ are what Miss Bunting saw and described as polar cells, which is a mistake that might be easily made. In *Pennaria* these papillæ push the false membrane away from the egg as they form and after being set free remain in this same structure for some time. The papillæ in both species are wholly devoid of chromatin and so far as could be determined entirely cytoplasmic phenomena.

FRAGMENTATION OF THE NUCLEUS, — AMITOSIS.

Fragmentation. — If by fragmentation of the nucleus is meant that the entire nucleus disappears and its contents disperse throughout the cytoplasm then I find no evidence of such a process in these hydroids. But what shall be said of the chromatin changes before maturation in *Hydractinia* and in *Pennaria* after maturation where large quantities of chromatin migrate into the cytoplasm never to return to the nucleus so far as one can determine? This certainly seems to be a kind of fragmentation. Why does it occur? Has the maturation phenomena failed to fully prepare the egg nucleus for fertilization? These are questions which those who would make the development of all eggs conform to a definite series of changes must explain.

Amitosis. — By this process it is understood that the nucleus divides without the chromatin passing through a complicated series of changes and without the formation of a spindle. Frequent search has been made for amitosis in these eggs but without finding any positive evidence. The irregular and claviculate shaped nuclei were critically observed and in every instance eventually either asters or the very characteristic chromatin changes were taking place in them. The mere shape of the nucleus in *Pennaria* is no indication of amitosis, nor is it necessary that the chromosome vesicles become transformed into the single "resting nucleus." *The cluster of vesicles which Hargitt frequently finds is not uncommon in my material but is interpreted in this paper as late telophase.* This is a point concerning which there may be a difference of opinion but taking all of the facts into consideration, these vesicles seem to me to indicate mitosis rather than amitosis.

INCLUSIONS IN *Pennaria*.

There are found in the eggs of *Pennaria*, even before maturation, bodies which for the lack of a better term are designated inclusions, Fig. 29, Pl. IV. Thus far they have not been seen in the segmenting egg. As many as three such inclusions have been discovered in a single egg. When newly formed, the substance of the inclusion takes the same stain as the surrounding cytoplasm, but in the older stages this contained substance stains faintly, eventually leaving a cavity. This cavity then is obliterated by the encroachment of the cytoplasm. The substance within the inclusions in appearance and staining reactions is certainly similar, if not identical, to the cytoplasm, and the whole structure looks like a food-vacuole in which cytoplasm is being digested. Their origin has not been determined. They can hardly be regarded as polar bodies, because they may appear before maturation begins.

SUMMARY.

1. In many particulars this work is a confirmation of the previous paper by Hargitt, especially in regard to the several nuclei in the unsegmented egg, the irregular shape of many such nuclei, and the irregular phases of cleavage.

2. The ova arise in any region of the polyp, which is contrary to Bunting's statements. The young ova gradually increase in size, during which time the nucleolus becomes vacuolated and the cytoplasm is occupied by numerous microsomes which become transformed into spherules. The cytoplasm changes its staining reaction during this time.

3. The chromatin during the growth of the egg stains less intensely than when in the younger state. Much of the chromatin migrates into the cytoplasm and is surrounded by vacuoles. The highly vacuolated condition of the cytoplasm is probably directly due to this migrating chromatin. The size of the nucleus decreases greatly.

4. In *Hydractinia* there were found three distinct kinds of granules, yolk masses, coarse granules and small bodies around the periphery. The small granules are distributed exclusively to the ectoderms.

5. Maturation begins in *Hydractinia* before the eggs are deposited. The process is by the formation of a distinct mitotic figure. It is very rare to find a polar body attached to the deposited egg.

6. The female pro-nucleus is very much smaller than the egg nucleus before maturation but it persists as a definite structure until cleavage begins. It is not at any time indistinguishable. The male pro-nucleus moves through the cytoplasm until it approaches the female pro-nucleus when the two fuse and fertilization is effected.

7. The first, and all subsequent cleavages, is by the mitotic process. A definite segmentation cavity is formed in the two-celled stage which increases in size. This cavity is gradually filled with cells until the planula is a solid mass of cells.

8. The false membrane in *Pennaria* is a transitory structure and probably of a fluid nature. Later its place is taken by a true membrane.

9. Maturation in *Pennaria* begins before the eggs leave the medusa. The polar bodies are ephemeral in character and rarely found attached to the deposited egg. The polar bodies are formed by the mitotic process.

10. After the two polar bodies are formed in *Pennaria* there is a distinct migration of a considerable amount of chromatin into the cytoplasm. The chromatin is transformed into vesicles which eventually are taken up by the cytoplasm. Sometimes these vesicles contain chromatin and persist for some time and may (?) divide mitotically, giving rise to a pseudosegmentation.

11. The spermatozoa may penetrate the egg before it is laid. The sperm head is transformed into a male pro-nucleus which moves through the cytoplasm toward the animal pole to unite with the female pro-nucleus.

12. Cleavage in *Pennaria* is at all times by the mitotic process. The chromosomes become transformed into vesicles during the late anaphase and early telophase. The vesicles may or may not unite into a definite "resting nucleus" before the next cleavage.

13. During cleavage in *Pennaria* there is a distinct centrosphere which contains granules with centrosome powers. This centrosphere is more conspicuous in *Pennaria* than in *Hydractinia*. The new prophase spindle arises within the old centrosphere.

14. Papillæ are found in both *Hydractinia* and *Pennaria* before segmentation, much as described by Hargitt.

15. A partial condition of fragmentation is seen in the migration of chromatin into the cytoplasm in both species.

16. No clear evidence of amitosis was observed.

17. Inclusions are frequently found in the egg of *Pennaria*.

October 20, 1908.

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POSTSCRIPT.

CHAS. W. HARGITT.

As supplemental to the foregoing paper it may not be amiss to add a few brief notes and comments. First, to the effect that it comprises an integral phase of work which has engaged the writer for many years, and which is still in progress. This particular feature was undertaken at my solicitation in the summer of 1906, as stated by the author. Second, at the same time I likewise turned over to my son, G. T. Hargitt, material for work along similar lines, a brief report of which has already been made. (*Science*, March 12, 1909.)

Smallwood's paper was completed nearly a year ago, but was at my request delayed, pending completion of further work of my own which was well advanced, and which it was intended should appear at the same time as intimately related thereto. The appearance of a brief note by Beckwith (BIOL. BULL., March, 1909), suggests the desirability of the issue of Smallwood's paper without further delay.

Aside from one or two points suggested by Beckwith I shall not undertake any detailed comments in this connection. Her rather matter-of-course dismissal of my presumed errors as to maturation with the remark that it was due "simply to the fact that eggs were not collected at the right time of day" is, to say the least, somewhat gratuitous! One does not usually follow investigations over a period of ten years without having taken *some* precautions against the more obvious sources of error. The fact is, I had long ago provided against that contingency. Again, her equally hasty dismissal of any question of methods of technique is without warrant. It was this more than any other one matter that proved an obstacle to satisfactory cytologic results. This I have called attention to in at least two of my more recent papers. And the precaution mentioned in the above paper by Smallwood as to this point was explicitly my own suggestion.

A brief comment as to the question of amitosis raised by both Smallwood and Beckwith must suffice for the *present.* In the first place I have never questioned the fact of mitosis in any of the cases under review, as the most cursory attention to my papers will show. Whether there be *amitosis* is purely a question of fact. Were my own results the only evidence it might very well be questioned. Facts adduced from almost every phylum of the animal kingdom are too well known at present to warrant further dogmatism on *a priori* or theoretical grounds. Whether my interpretation of the significance of the nuclear and chromatin fragmentation and the vesiculate "nuclear nests" may be warranted I shall defer for later consideration.

EXPLANATION OF PLATES.

EXPLANATION OF PLATE I.

FIG. 1. Egg nucleus with small amount of cytoplasm to show the migration of the chromatin. *Nl′*, nucleus; *Nll′*, nucleolus; *chr*, chromatin.

FIG. 2. Portion of the cytoplasm and nucleus. The chromatin is mostly in vacuoles. *Nl′*, nucleus; *chr*, chromatin.

FIG. 3. Prophase first maturation. The walls of the nucleus are still intact. The egg is within the gonophore. *E. Nl′*, egg nucleus; *w. g*, wall of gonophore.

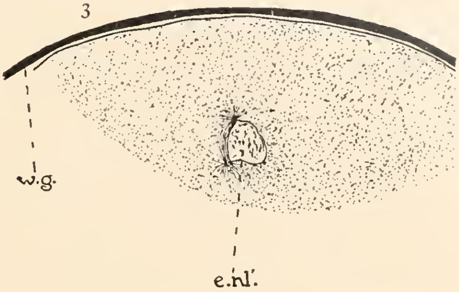
FIG. 4. Telophase of the second maturation. Egg deposited. *Cl. pol*^{2}, second polar body; *vsl. chr'so*, chromosome vesicles.

FIG. 5. Female pronucleus showing the remains of fibers that have persisted. Pronl. ♀, female pronucleus. *pr. gr*, peripheral granules.

FIG. 6. The approach of the male pronucleus. The female pronucleus is in the early prophase. Pronl. ♀, female pronucleus; pronl. ♂, male pronucleus.

FIG. 7. Prophase of third cleavage shows an elongated nucleus with asters and forming spindle fibers.

3


w.g.
e.nl'.

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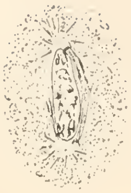

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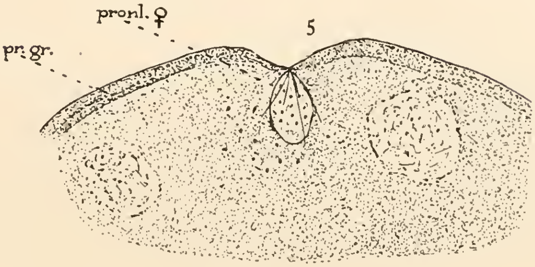
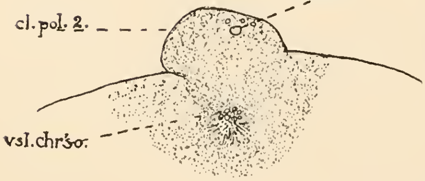

pronl. ♀
pr. gr.

4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cl. pol. 2.
vsl. chr'so.

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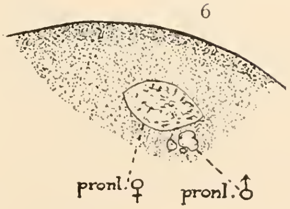
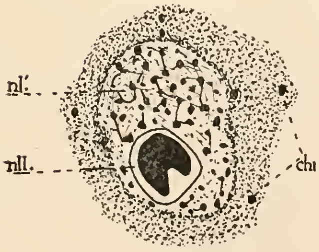
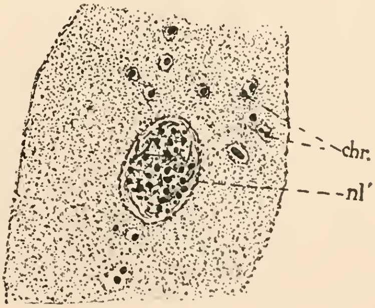

pronl. ♀
pronl. ♂

1


nl'.
nll.
chr

2


chr.
nl'

EXPLANATION OF PLATE II.

FIG. 8. Is taken from one of the cells shown in Fig. 9. The vacuolated condition of the cytoplasm is shown in the clear spaces. The spindle is in the anaphase and so directed as to set a cell free in the segmentation cavity. There is a large centrosphere at each pole.

FIG. 9. Outline drawings of embryo. Numerous vacuoles are present in the cells. *Seg. cav*, segmentation cavity.

FIGS. 10 to 29 are from *Pennaria*.

FIG. 10. Telophase second maturation. Egg in medusa. *Cl. pol. 1*, first polar body; *cl. pol. 2*, second polar body.

FIG. 11. Female pronucleus drawn from an egg before deposition. Pronl. ♀, female pronucleus.

FIGS. 12 to 18 show the migration of the chromatin from the pronuclei into the cytoplasm. Pronl. ♀, female pronucleus; ♂, male pronucleus.

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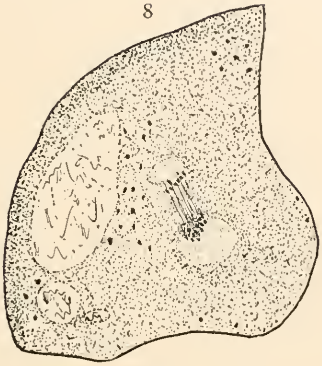

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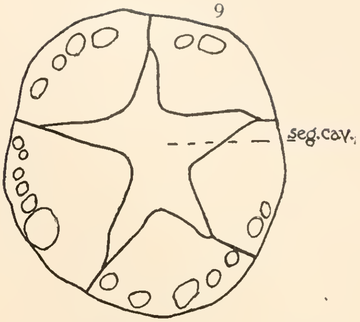

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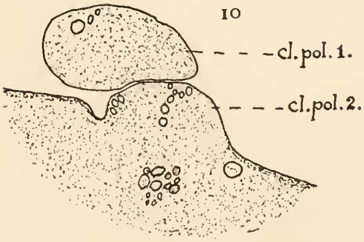
cl.pol.1.
cl.pol.2.

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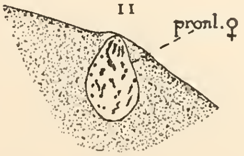

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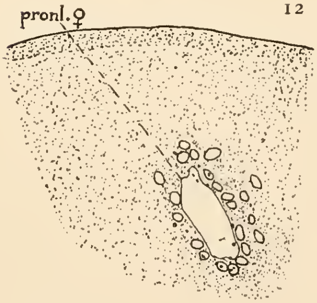

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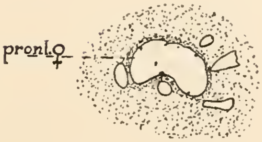

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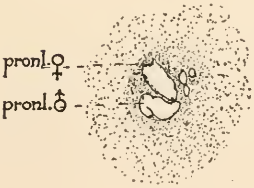
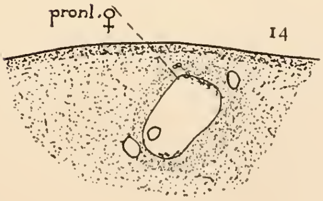

pronl.♀
pronl.♂

14



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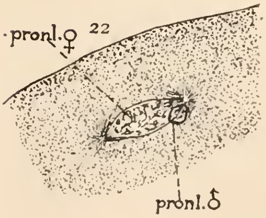
pronl.♀
pronl.♂

EXPLANATION OF PLATE III.

FIG. 19. Union of the male and female pronuclei. Pronl. ♀, female pronucleus; pronl. ♂, male pronucleus.

FIG. 20. Low power drawing of entire egg. The fine peripheral granules extend clear around the egg. Several nuclear-like bodies are interpreted as polyspermia.

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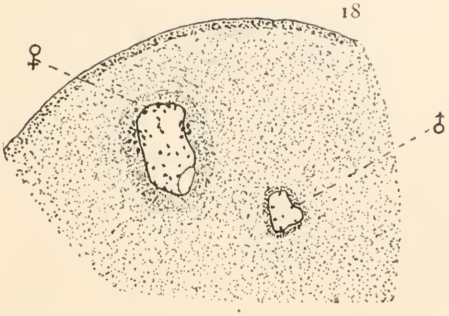

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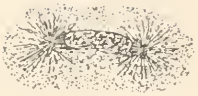

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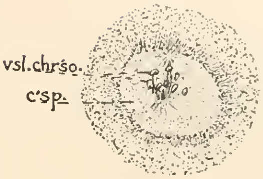
vsl.chrso.
c'sp.

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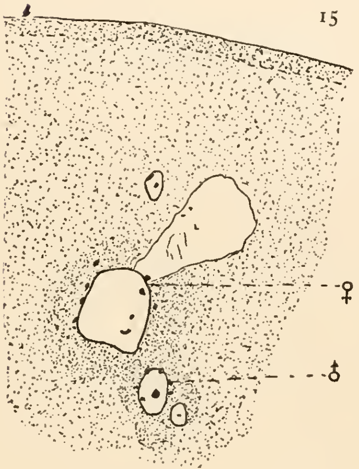

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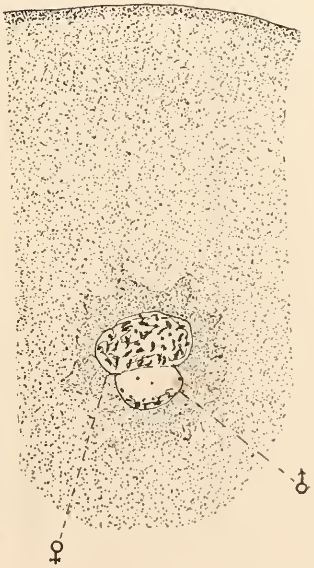

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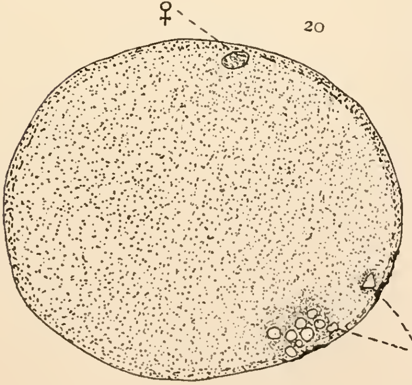

EXPLANATION OF PLATE IV.

FIG 21. Shows the presence of several nuclei in the unsegmented egg, one of which is probably the female pronucleus, pronl. ♀.

FIGS. 22 to 24. Three forms of the prophase in cleavage. Fig. 22, conjugation of pronuclei and formation of first segmentation spindle. In Fig. 23 the chromosome vesicles have not united. Pronl. ♀, female pronucleus; pronl. ♂, male pronucleus; *vsl. chr'so*, chromosome vesicles; *c'sp*, centrosphere.

FIGS. 25, 26, 27. Show the appearance of the segmentation spindle, migration of the chromosomes, and presence of the centrosphere; *c'sp*, centrosphere.

FIG. 28. Inclusions in the cytoplasm.

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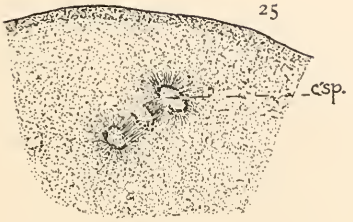

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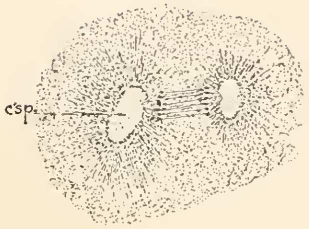

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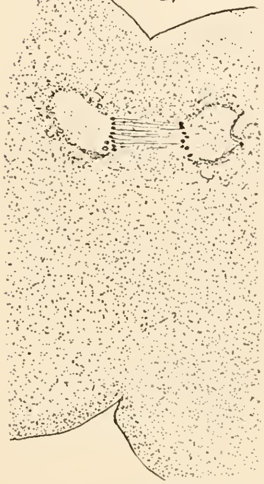

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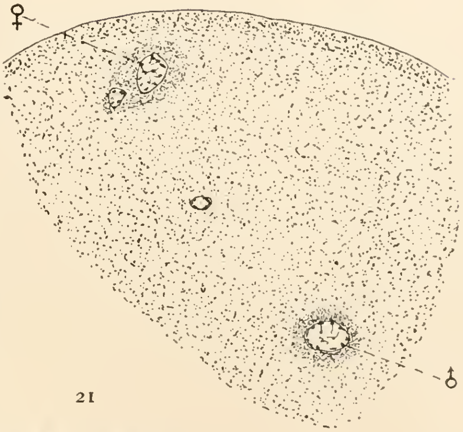

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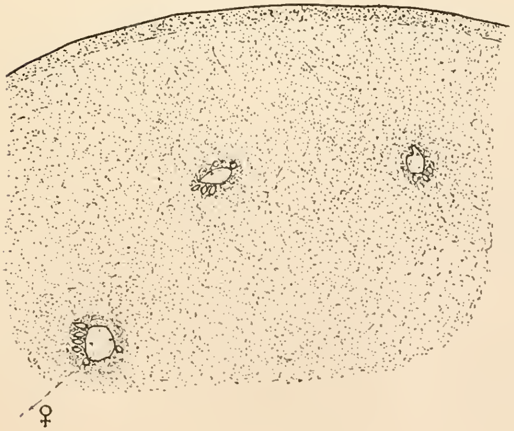

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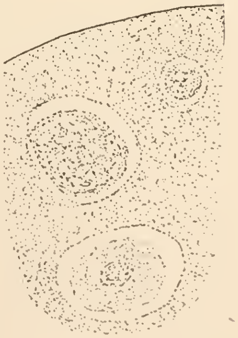

NEW OR LITTLE KNOWN PERMIAN VERTEBRATES. PARIOTICHUS.[1]

S. W. WILLISTON,

University of Chicago.

The University of Chicago Expedition to the Permian of Texas during the autumn of 1908 was very fortunate in finding a skeleton of a small reptile enclosed in a nodular matrix, probably the most complete of any specimen hitherto obtained from that formation. It is of especial interest since it gives, for the first time, a natural skeleton of a cotylosaur with all its bones in anatomical relations, scarcely a single one disturbed by extraneous force in fossilization. The original nodule measured about six inches in its greater, by five in its lesser diameter, and about two inches in thickness. The nodule, as discovered, was split horizontally, the thicker portion enclosing most of the skeleton lying upon its back; the thinner with portions of the bones partly enclosed in it, and with the right front leg almost wholly so. One small piece of the thicker side, and a yet smaller fragment of the thinner were not recovered. The missing portions, however, are of minor importance, and are in part supplemented by the two blocks. The specimen was discovered on a gently sloping surface near the Wichita River, north of Mabelle, by Mr. Paul Miller.

The material of which the nodule is composed is a rather hard argillaceous limestone, and has necessitated very patient labor on the part of Mr. Miller, with awl and needle, in its preparation, many of the bones being so small as to require the use of a magnifying glass. The skeleton, which measures nearly fifteen inches in length, is closely coiled, the tip of the tail lying under the front extremity of the skull. As exposed on the two blocks, the hyoid bones are in place; the pectoral girdle is very slightly displaced, with both arms articulated; the right arm is strongly flexed at the elbow, with its outspread hand underlying the posterior part of the right mandible; the left arm is extended backward close to the vertebral column, its fingers protruding from the edge of the block and for the most part lost; both hind legs are articulated throughout, turned backward by the side of the tail, and not a single bone seems to have been lost or disarticu-

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[1] "Cotylosauria," *Journal of Geology*, XVI., p. 139; *Lysorophus*, this journal, XV., p. 229; *Diplocaulus*, *Trans. Kansas Acad. Science* (in press); *Trematops*, *Journal of Geology*, XVII. (in press).

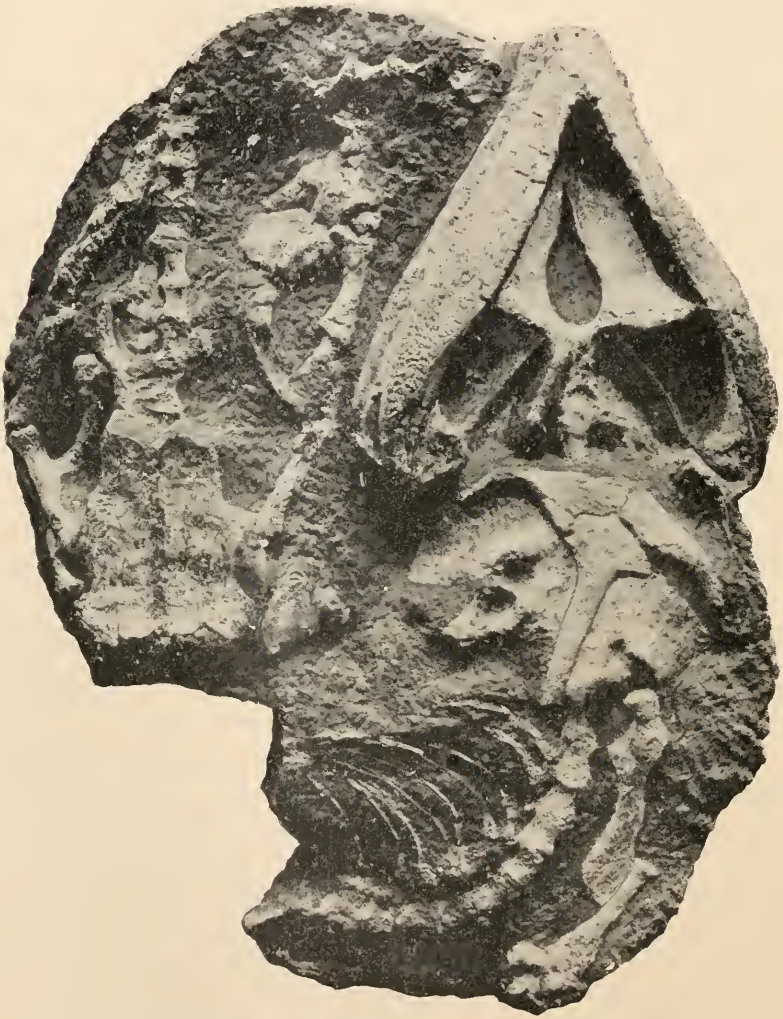

FIG. 1. *Pariotichus laticeps* Williston. Photograph of specimen, from the thicker half of nodule; natural size.

lated; both the feet, unfortunately, are lying in part upon their fibular side, concealing some of the bones; the tenth to the thirteenth caudal vertebræ are disarranged and partly missing, probably due to the fact that they lie partly over the right foot; the small terminal vertebræ of the tail are also missing, where they protruded from the margin of the slightly eroded nodule. On the whole, the only doubtful details of the skeleton are the number and arrangement of the tarsal bones, and the extreme tip of the tail. The extreme terminal phalanges of the first three fingers, because of their minute size, may have been destroyed in the preparation of the block, or slightly dislodged.

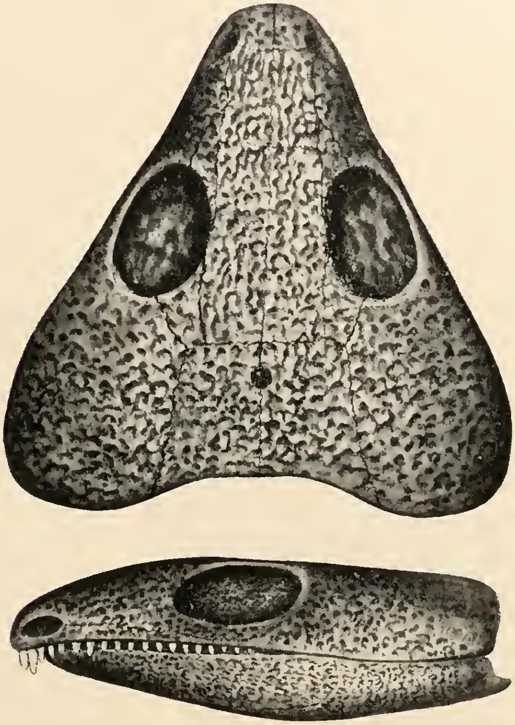

FIG. 2. *Pariotichus laticeps*, skull from above and from the side; natural size.

Skull (Figs. 1, 2). — The skull is in a marvellously perfect condition, the only injury it has suffered being a very slight erosion of the extreme tip of the muzzle where it protruded from the edge of the nodule. It is remarkable among reptiles for its great width and depression, its width posteriorly being fully equal to its length. The pitting of the upper surface is small and reticulate, with a slight, though distinct, indication of longitudinal ridges. The parietal and frontal bones posteriorly are slightly concave in the middle. The nares are small, situated nearly at the extremity of the muzzle, oval in shape and directed more outward. In front of the orbits the face is a little constricted, with the sides more strongly convex. From the front of the orbits the lateral lobe of the cranium is convex outward to about midway between the orbit and the hind border, where the curve is slightly inward. There is a rather strong emargination of the hinder border of the cranium in the middle, with the outer third on each side gently convex or nearly straight. The large orbits are located a trifle in front of the middle of the skull. They are oval in outline, with the long diameter antero-posterior, measuring eighteen millimeters in length by fifteen in breadth. The plane of their margins is directed a little forward and upward at an angle of about 45°. The large pineal foramen is nearly midway between a line drawn through the posterior margin of the orbits and the hind border of the skull in the middle line. The sutures of the skull are, for the most part, indicated by very delicate lines, requiring a hand lens to follow. I have given such as I feel sure of. The plane of the upper surface of the skull is nearly horizontal as far forward as the front end of the orbits, whence it slopes gently to the front extremity, with a slight convexity. The mandibles are in position on the under side, slightly pushed to the left. The symphysis is short, the rami rather narrow in front, their outlines very much like those of the side of the cranium, curving inward posteriorly. They are broadest and deepest a little back of their middle, or opposite the transverse bones, which abut against them. Distinct sutures for the splenial, dentary and angular are seen anteriorly on the outer side. The dentary ends by a broad curve upward, with the slender prolongation of the angular enclosed between it and the margin of the splenial below. At the hind extremity the small, inwardly curved angular process is visible. The slight lateral pressure upon the mandibles has left exposed the insertion of the maxillary teeth of the right side, but the very delicate teeth themselves have suffered in the preparation, though their roots are clearly to be seen. On the premaxillary there are three stout teeth, the largest of the series of either jaw. Of these the third is the smallest, the first and second of nearly equal size, judging from their roots. Back of the premaxillary teeth, in addition to the roots seen on the right side, the teeth themselves are preserved on the left side, crowded rather closely upon the mandible. They are all rather small, the fifth or sixth of the series, counting the premaxillary teeth, about a third of the distance to the orbit back of the narial opening, is the largest. They are all rather obtusely pointed, and are separated by spaces about equal to or somewhat less than the width of the teeth themselves. In a space of ten millimeters back of the largest maxillary tooth there are five teeth. The mandibular teeth cannot be made out. It is altogether probable that there are additional teeth in secondary rows upon the maxilla and dentary, but the close union of the mandibles with the cranium prevents their detection.

The palate is very fully exposed and is undistorted. The internal nares, placed far forward, are above the mandibles and yet concealed by the matrix. The broad, flat palatines and vomers — for sutures are nowhere determinable — diverge very gradually to about midway between the mandibular symphysis and the basisphenoid, where they separate more widely, leaving a rather large ovate space, yet filled in with matrix. I can distinguish no presphenoid in this ovate space, but it is possible one exists directed obliquely toward the roof of the skull. Along the margin of these bones by the side of the ovate opening and anteriorly are one or two rows of minute tubercular teeth, and, just in front of the descending convexity of the transverse, there is an oblique row of six or seven palatine teeth on each side. The lateral palatal plates descend posteriorly in a convex surface, to nearly the lower margin of the mandibles, with a thinned, convex posterior margin. The lower convexity of these transverse bones (for they are doubtless separate elements, though they have never been suturally distinguished) is covered with a patch of tubercular teeth. The union of the pterygoids with the basisphenoid is very evident in the constriction at either side of the front of the basisphenoid, but the suture is not determinable. From this constriction and union with the short basipterygoid processes of the basisphenoid, the posterior plates of the pterygoids diverge to the inner side of the quadrates, opposite the inner angle of the articular bone of the mandible. These plates have a thin, straight, horizontal lower margin, whence they slope inwardly and upwardly into a somewhat concave surface, narrow and nearly vertical in front, widened behind, leaving a small oval space anteriorly between them and the basisphenoid. The basisphenoid is narrow in front, gently widened behind, shallowly concave in the middle and limited on either side by a rather rugose ridge. From near the posterior, somewhat divergent, ends of these ridges, a slender bone runs outward and backward by the side of the inner margin of the pterygoid plate. It is in all probability the stapes. Upon the whole, the structure of the palate and occipital region is quite like that of *L. hamatus*, which will be fully described and illustrated in a future paper from a remarkably perfect specimen in the University collections. Lying under the palate were two slender rods. One of these has been necessarily destroyed in getting to the palatal surface; the other still remains in the matrix. They nearly meet in the middle anteriorly, just back of the interpterygoid vacuity, diverging posteriorly nearly parallel with the margins of the pterygoid plates to near the quadrate. Back of these and articulated with them are a pair of more slender bones bent inwardly near their middle, and terminating acutely behind, nearly parallel with the front margins of the clavicles.

Vertebræ. — The vertebræ are united in a continuous series from the skull to near the tip of the tail, forming a U-shaped curve. The pectoral girdle still conceals the anterior five or six of these and the posterior seven of the presacral series were lost in the missing fragment of the nodule. However, the top of the nodule over these missing seven vertebræ has preserved in part impressions of them, and, inasmuch as the vertebræ themselves, where exposed, are all of precisely the same length, seven and a half or eight millimeters, the number of presacral vertebræ in the entire series is determinable with but slight chance of error. This number was either twenty-three or twenty-four. Broili has determined the number of presacral vertebræ in *Labidosaurus hamatus* as either twenty-four or twenty-five; twenty-three or possibly twenty-four is the number of presacral vertebræ in the rhachitomous *Trematops* as determined by me; twenty-three or twenty-four was the number ascertained by Thevenin in *Sauravus costei*, a probable reptile from the Upper Carboniferous of France; and I believe that *Isodectes Copei*, from the Lower or Middle Mississippian of Linton, Ohio, had the same number. This uniformity seems to be more than a coincidence. Of this number the atlas alone can be called, as in the amphibians, a true cervical. All the vetebræ bore ribs, and, inasmuch as there was no sternum in these early forms, a distinction into neck and trunk is impossible. The centra of our specimen are rather slender — more so than in *Labidosaurus* — each with a marked constriction in the middle. Some of the posterior vertebræ lie partly upon their sides, disclosing the attachment of the ribs. No distinct process is seen on the centrum for them, differing in this respect from *Labidosaurus*, though doubtless the lower part of the proximal extremity of the ribs did articulate with the centrum. A small intercentrum is present in one or two of the anterior vertebræ, and a small space is seen between the lower edges of the centra of all. The sacral vertebræ are of course not visible in the pelvis. There were, in all probability, but two sacral vertebræ, though this is not certain since another form of Permian reptile, which will be illustrated and described later, has three, while *Labidosaurus* has but two. If the vertebræ above the pubes and ischia are of the same length as those immediately following or preceding, there are six concealed from view, one of which is the first presacral, and three the basal caudals. Behind the pelvis eight caudal vertebræ in a connected, gently curved series are visible. They are somewhat shorter and more slender than the presacral vertebræ, the entire series measuring about forty-six millimeters. Beginning with the second of these there are long, slender chevrons, each reaching to beyond the end of the next succeeding centrum, that is, about fourteen millimeters in length. Just how far back these chevrons continue is not certain, but at least eight centra bore them. The first entire vertebra back of the pelvis bears a long, curved rib on each side, springing from the anterior end of the centrum. The next two vertebræ doubtless have similar, but short ribs, though only the proximal ends of them are seen. I do not think that the following vertebræ bore ribs, or, if so, they were much shorter. I can find no indications of such. In the restoration, these caudal ribs are shown directed backward, as in the matrix. Doubtless in life they were directed more downward, indicating a thick basal portion of the tail, probably compressed from side to side. Beyond the eighth visible caudal there is a break in the series, the only indication of contemporary extraneous force shown in the entire skeleton ; and this may have been due to the fact that this part lies somewhat under the right hind leg in the nodule — that is, if the skeleton was originally fossilized in a prone and not supine condition. Following this gap there are three articulated vertebræ in line with the curvature of the basal portion of the tail. They are more slender than the preceding ones and clearly lie in their original position, the intervening five vertebræ having been dislodged, fragments of which are still preserved near the break. The extreme tip of the tail came near the margin of the nodule, at or below the tip of the muzzle, and has been destroyed by the erosion of the nodule. Perhaps a half dozen very small vertebræ are missing here, making altogether about twenty-five vertebræ for the number in the tail, or for the entire column about fifty, with a possible error of three or four more.

The dorsal ribs have an expanded proximal end, but without distinct differentiation into head and tubercle. The first one visible is attached to the vertebra concealed in part by the caudal end of the interclavicle, that is, the seventh or eighth of the series. It is altogether probable that the first four or five ribs were shorter, with expanded distal ends, as in *Labidosaurus*. From the eighth to the seventeenth the ribs lie nearly parallel on the right side, on the left being crowded more together at their ends. The longest of them measure forty-six millimeters along their considerable curvature. They are slender throughout. The broken ends of the eighteenth and nineteenth are visible in the matrix, but little if any shorter than the preceding ones. A fragment of what should be the twenty-first or twenty-second is also visible on the right side, but its length is not determinable. I have, therefore, shaded the last four ribs in the restoration, and it is possible these vertebræ were entirely ribless, as I have decided they were in *Labidosaurus*.

Pectoral Girdle. — The pectoral girdle lies almost perfectly in position, the hind end of the interclavicle only, turned slightly to the right, and the right coraco-scapula pushed slightly forward, or the left one backward. It is very certain that the girdle was attached immediately back of the skull, the front part underlying the occipital condyle even. In structure it is almost identical with that of *Labidosaurus*, as figured by me. The position in which they lie has slightly separated the clavicles at their scapula, attachment. I find no indications of a cleithrum. It is very evident that the coracoids in life were in immediate contact along the median line, covered over by the prolongation of the interclavicle. The scapulæ curve upward at an angle of about forty-five degrees from the plane of the coracoids. Possibly this angle has been reduced slightly by pressure, but I think not. The scapulæ are directed, not backward, as has been supposed, but obliquely upward.

Front Legs. — The humerus is of the usual shape, expanded proximally and distally in planes meeting each other at an angle of sixty or seventy degrees. The bone, like all other parts of the appendicular skeleton, is distinctly more slender than in *Labidosaurus.* The radius is a rather slender bone, cylindrical at its proximal, flattened and expanded at its distal extremity. The ulna, much broader and thicker at its proximal end, has a distinctly produced olecranon, and the curvature of the rather slender shaft in the middle is distinctly away from the radius. The most of the forearm and foot of the right extremity are preserved in the thinner block, the proximal ends of the radius and ulna in the thicker close by the right pubis. The hand, as thrust forward below the right mandible, is outspread and fully articulated, the middle of the wrist somewhat depressed by the angle of the mandible in the mud, slightly turning the distal end of the ulna. The terminal phalanges were probably present or but slightly dislodged, but their minute size has made it almost impossible to detect and work them out from the hard matrix. The carpus clearly agrees in its chief features with the carpus of *Labidosaurus* "*incisivus*," as figured by Case and myself, save that the parts were reversed in the figures. I give herewith a better figure of the labidosaur carpus, which has been more completely removed from its matrix (Fig. 4). Because of the compression of the middle part of the carpus in our specimen, it is impossible to be quite sure of the presence of both centralia. The radiale is much broader than long, articulating with the radius, the distalia of the second and third digits, and with one or possibly two centralia. The ulnare is a much larger bone, articulating proximally with the ulna, distally with the two inner distalia, and on the outer side with the intermedium. That there is a free intermedium here as in *Labidosaurus* is certain, but I cannot be quite sure of its extent, a part of it being apparently covered over by the radius. It articulates, as in *Labidosaurus*, proximally with the ulna and distally with a centrale. Five digits are present, as was to be expected. The first is represented by its metacarpal only, either slightly removed from its articulation with the radiale, or, what is more probable, with its distale lost, or cartilaginous. In the restoration it is shown removed from the carpal bones as in the photograph of the hand also given herewith (Fig. 3). The first metacarpal is the shortest of the five, and is only moderately ex-

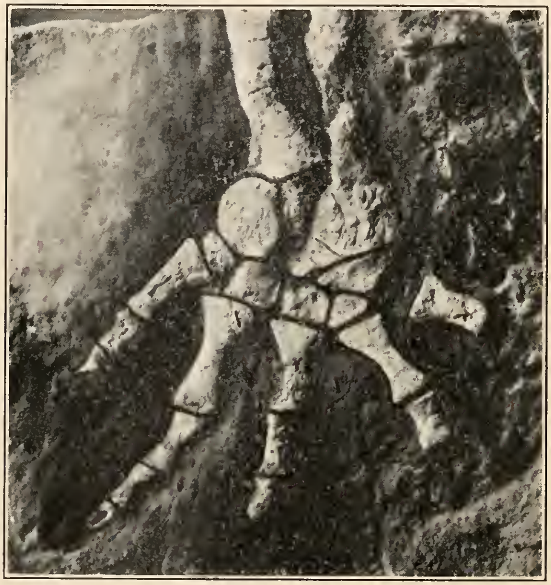

FIG. 3. *Pariotichus laticeps*. Photograph of right front foot, from thinner half of nodule; natural size.

panded distally. No phalanges are preserved. The second metacarpal is much longer than the first, and is much constricted in its middle. It has one short phalanx articulated with it, but little more than half the length of the metacarpal. Additional phalanges are not preserved, but, from its size, it seems very probable that two more, and not more than two, were originally present. The third metacarpal is much like the second, but is a little longer. Two phalanges are present, the first about two thirds the length of the metacarpal; the second fragmentary. There may have been a third, ungual phalanx present. The fourth metacarpal is the longest and stoutest of all, its proximal articulation more oblique than is the case with the preceding one. The first phalanx is about three fifths the length of the metacarpal. The second phalanx, much shorter and smaller, has at its tip a small fragment. There may have been a fourth phalanx, though there is not much probability of it. The fifth metacarpal is a little shorter than the fourth, somewhat curved and more slender. It has a small and short proximal phalanx and a fragment of a distal one at its tip. In all probability there were no more. It is, it is seen, impossible to say with certainty what the phalangeal formula of *Pariotichus* was, save that quite surely it was not that of the modern lizards and *Sphenodon*, 2, 3, 4, 5, 3. In much probability it was 2, 3, 3, 4(3), 2.

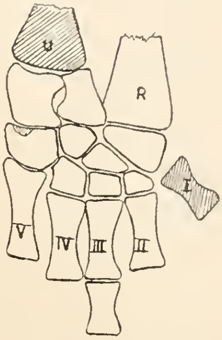

FIG. 4. *Labidosaurus.* Carpus ; one half natural size.

Pelvis. — The under side of the pelvis lies in perfect preservation and position. It is, like the pectoral girdle, of the "old fashioned" type, elongate and plate-like, without thyroid foramen. The two sides meet in a long median symphysis, closely applied, but not sutural. The pubes, broadest in front, have a slight emargination in the middle in front. The small pubic foramen lies near the acetabulum, at the junction of the first and second thirds of the combined bone. Just inside this foramen, and a little to the outer side of the middle of each pubis, a pronounced thickening or ridge runs forward to the anterior margin, forming a shallow fossa on each side of it. The pubo-ischial suture is, apparently, a little back of the pubic foramen, running transversely across. It is not at all distinct, and I am not sure of it. Lying in the median emargination of the pubes there are three or four very slender ventral ribs, and by their side, a fragment of what appears to be a thin plate, which may have articulated with the thickening of the front margin before mentioned, a thickening characteristic of all the smaller cotylosaurs, apparently. The pelvis, so far as it can be seen, is almost identical with that of *Labidosaurus*, and other Permian cotylosaurs, forms of which will be figured later.

Legs. — As stated, the hind legs are both preserved, trailing backward from the acetabulum, both of them somewhat bent at the knee. The femur of the right side suffered somewhat in the fracture of the nodule, and is only partly preserved. That of the left side, so far as it can be seen partly embedded in the matrix, agrees quite with that of *Labidosaurus*, though more slender. The tibia, also shown best on the left side, offers nothing unusual. The fibula, hitherto unknown in the cotylosaurs, is strongly curved, with a considerable expansion at its lower extremity, and with a small, rounded upper end. The feet, unfortunately, have both been preserved lying more or less on their fibular side, and in consequence with the toe bones more or less concealed. However, it is quite certain that no force was brought to bear upon them to displace them, save that of their own weight. The lower leg of the right side and the tarsus are spread out flatly, but with the digits piled upon each other. A large, flat fibulare articulates with the fibula of the right side in position, closely articulating on the inner side with another large bone, evidently the united tibiale and intermedium. Four tarsal distalia are visible. The shapes of the bones distinguished agree in general so well with those of *Labidosaurus* as figured by me, that I have no hesitation in giving the others from the same genus, shaded in the figure. The tibiale, however, must have been shorter than in *Labidosaurus*. As regards the toes, all five metatarsals are visible on one or the other side, and many of the phalanges, save those of the fifth toe. In the figure given in the restoration, the unshaded phalanges of the other toes are given precisely in the positions they occupy with regard to the tarsus, so that the length of the toes is quite certain. Those phalanges which cannot be extricated from the matrix are shaded. In all probability the phalangeal formula is like that of the front feet ; certainly there cannot be a greater number.

Six genera of Permian reptiles, all from Texas, are referred by Cope to the family Pariotichidæ ; and, notwithstanding the difference in the teeth, I am disposed to add the seventh genus, *Labidosaurus*, to the same family. They are defined by Cope as follows :[1]

1. Teeth on the maxillæ and mandibles in a single series.........*Labidosaurus* Cope.
   Teeth in more than a single series..2.
2. External nostrils inferior ; mouth posterior in position ; mandible short and with a few acute teeth.........................*Hypnopous* Cope.
   External nostrils lateral..3.
3. Palatal and splenial teeth with compressed crowns..........................4.
   Palatal and splenial teeth obtuse, forming a grinding pavement ; median maxillary and anterior incisor teeth enlarged..........................*Pantylus* Cope.
4. Teeth equal, acute..........................*Isodectes* Cope.
   Teeth increasing gradually in length anteriorly....................*Captorhinus* Cope.
   Teeth enlarged in the middle of the maxillary and anterior part of the incisor series..........................*Pariotichus* Cope.

The genus *Helodectes*, provisionally placed in the Pariotichidæ, is distinguishable by the two rows of teeth on the jaws, the "bases of which are wide ovals, transversely placed."

Isodectes is figured by Cope as having the prefrontals and postfrontals meeting broadly over the orbits, widely excluding the frontals from the orbit. The skull, moreover, is much longer than broad in the type species, *I. megalops*. *Pantylus* also has the prefrontals and postfrontals meeting broadly as in *Isodectes* in the type species, *P. cordatus*, which, moreover, has a rounded muzzle, and is widely expanded posteriorly. *Captorhinus* has an elongate, pointed skull, with the orbits twice the diameter of the interorbital space. *Hypnopous* is wholly out of consideration because of the remarkable position of the nares. Assuming that our species has more than one row of teeth on maxillæ and mandibles, its exclusion from the labidosaurs is of course evident. As that character cannot be determined save by the mutilation of the otherwise perfect skull, the doubt must be left. By ex-

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[1] *Proc. Amer. Phil. Soc.*, XXXIV., 1895, p. 445.

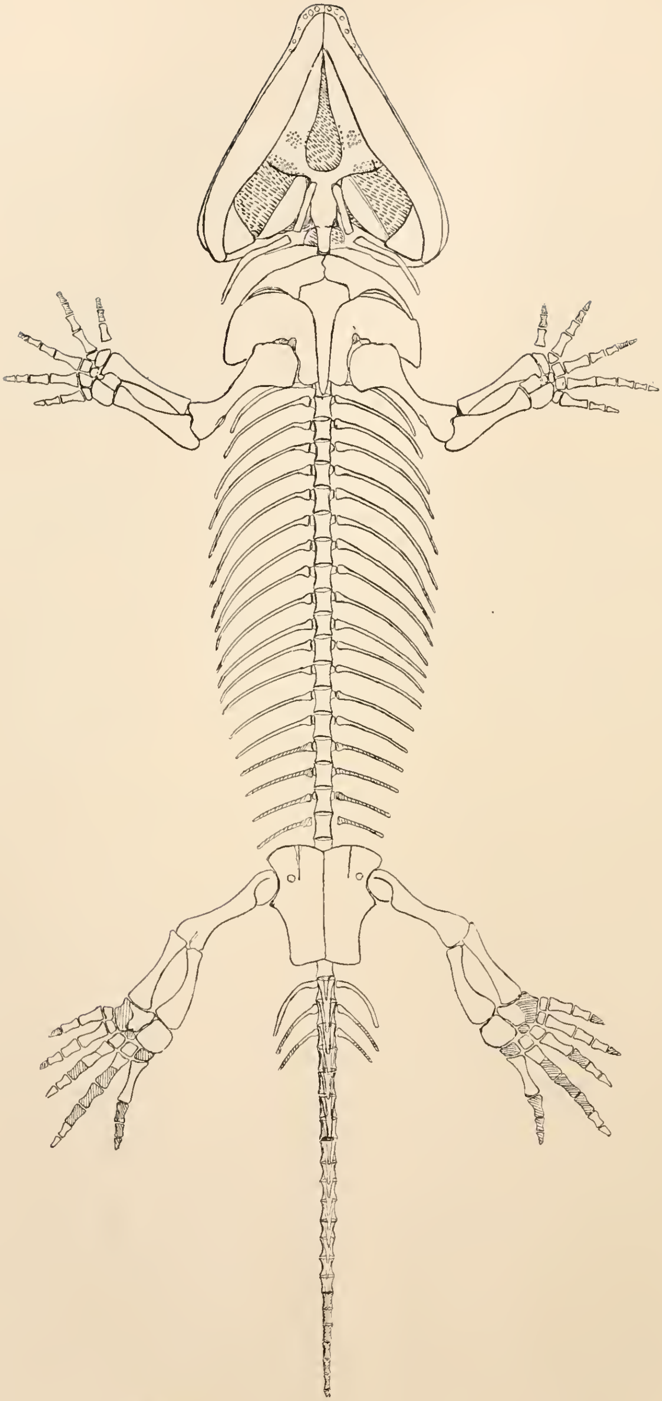

FIG. 5. *Pariotichus laticeps.* Restoration of skeleton ; one half natural size.

clusion, we have only the genus *Pariotichus* left, and in all its characters, so far as they have been developed in the known species of the genus, the agreement seems sufficiently certain. Six species of *Pariotichus* have been described. Of these, *P. brachyops* is excluded by the large maxillary tooth not being below the anterior border of the orbits, but much further forward, by the relative size of the orbits, etc. *P. aguti* is easily distinguished by the elongate shape of the head, its less depressed form, etc. *P. isolomus* differs distinctly in its less expansion posteriorly, its length being distinctly greater than its width, and the absence of a posterior emargination of the cranial border. *P. incisivus* has been wrongly identified as having a single row of teeth on mandibles and maxillæ by both Case and myself, whereas Cope distinctly figures it (*Trans. Amer. Phil. Soc.*, 1886, p. 290, Pl. II., Figs. 4 and 5) as having additional teeth. The form described by me as *Labidosaurus incisivus* is therefore something else. *P. incisivus* is described and figured by Cope as having a purely reticulated sculpture of the skull, which he accepts as of specific value. Our species has the sculpture, distinctly longitudinal, on the upper side of the skull anteriorly at least. *P. aduncus* is distinguished by the size of the orbits, etc. We have, then, but a single species left, *P. ordinatus* Cope, described from such scanty material that it is doubtful if an actual comparison of the type specimen would resolve doubt as to its identity with the species herein described. Our species may, therefore, be provisionally given the name of *P. laticeps*.

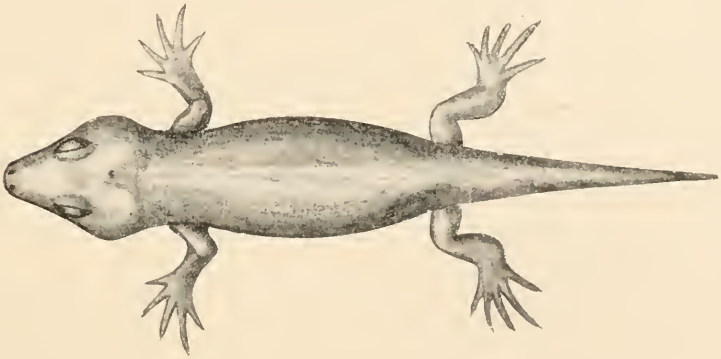

FIG. 6. Life restoration of *Pariotichus laticeps*; one fourth natural size.

Vol. XVII *September, 1909.* *No. 4*

BIOLOGICAL BULLETIN

SPERM-TRANSFER ORGANS IN CAMBAROIDES.

E. A. ANDREWS.

Amongst the arthropods it is not uncommon for some of the limbs to be used for transferring the sperm from the male to the female. In some of these cases the path of the sperm from the male to the egg is very complex, and we may speak of "indirect sperm-transfer."

Thus in the common crayfishes of the United States, of the genus *Cambarus*, the first and the second limbs of the abdomen of the male are special organs that we will call the first and the second stylets, which conduct the sperm from the male openings upon the bases of the fifth pair of walking legs to the surface of the female. In the female the sperm is received into a pouch in the shell on the under side, between the fourth pair of walking legs. This sperm receptacle is hollowed out in the so-called annulus, or special sternal plate, and in this pouch the sperm remains till the eggs are laid. In the crayfish of Europe, however, the sperm conveyed by the stylets is deposited freely over the sternum, in secreted tubules, or spermatophores, and there is no sperm receptacle.

There are then two forms of indirect sperm transfer within this family, in the two genera *Cambarus* and *Astacus*.

The crayfish of Japan and the Amoor River region are so different from other species of *Astacus* that it is a question whether they do not form a distinct genus. They resemble *Cambarus* so much that Faxon called them *Cambaroides*. Nothing is known as to the method of sperm transfer in this *Cambaroides* subgroup of *Astacus*, but the following account of the anatomy of the organs concerned may aid in a tentative view as to what actual observation of the process may reveal.

The material used was kindly loaned by the National Museum, and consisted of a very few specimens of *Astacus* (*Cambaroides*) *similis* from Corea and some ten specimens of *Astacus* (*Cambaroides*) *Japonicus* from Hakodate, Japan. These last were obtained by the "Albatross" from the market in July, 1906, and were remarkable in being all strung along upon bits of stick that had been thrust through several crayfish, one after the other, passing through the head-thorax and abdomen lengthwise. From the condition of internal anatomy the specimens would appear to have been dried before they were preserved by the naturalist.

Observations upon *Cambarus* have shown that when the stylets are being used to fill the sperm receptacle the male is firmly fastened to the female by two pairs of hooks, or spines, that stand out like spurs from the walking legs and are carefully fitted into the groove between the segments of the legs of the female. In fact, experiments show that without these spines the other organs are of no avail as the male is not able to transfer the sperm to the sperm pouch. In *Cambarus* there are then three sets of necessary external organs concerned in sperm transfer, the hooks and the stylets of the males and the sperm receptacles of the females.

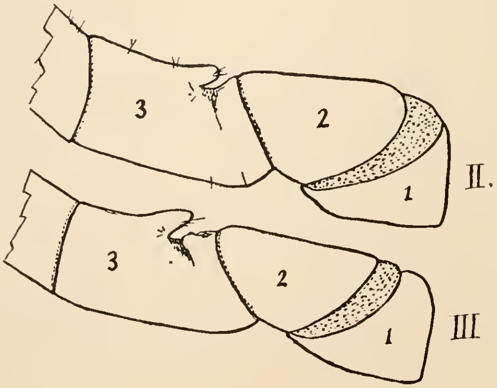

FIG. 1.

In *Cambaroides* the hooks are present as well as the stylets but in the females there is no discovered receptacle though the annular plate is somewhat modified.

We will describe these three sets of organs, in the following order: hooks, stylets, annular plate of female.

In *Cambaroides similis*, a specimen 55 mm. long had the hooks developed as in Fig. 1, a blunt rounded spine upon the third segment of the second and the third walking legs. Each spine bears a few setæ. They are like the spines of *Cambarus Montezumæ* in being on the second and third legs, but are much shorter, more blunt and less effective as hooks. The spine of the third leg is the better developed.

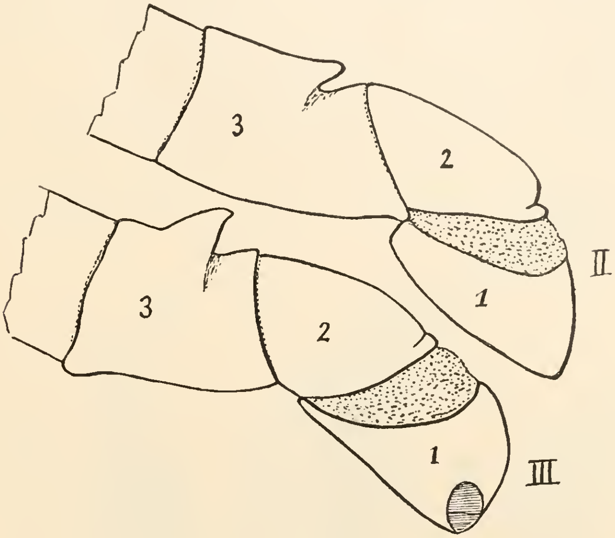

Fig. 2.

In *Cambaroides Japonicus*, Fig. 2, the hooks are essentially the same, but more pointed. In both cases the resemblance of the hooks to those used by *Cambarus* is so strong that one would infer that they are probably functional in *Cambaroides*.

In comparing the stylets of *Cambaroides* with those of *Cambarus* we note that the first lacks the fine detail of apex commonly found in *Cambarus* and is a more stout and undifferentiated organ. In *Cambaroides similis*, Fig. 3, the first stylet is a clumsy cylinder having a movable joint between the long protopodite and the somewhat longer distal endopodite.

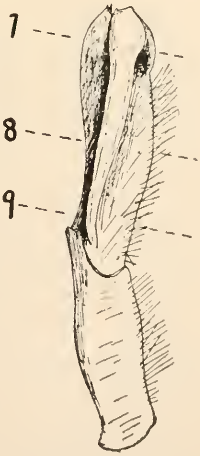

Fig 3.

Both bear a fringe of setæ along the external edge. This long line of setæ seems to represent what is found upon the edges of the common pleopods and may be regarded as a mark of little specialization or of retention of primitive characters, since in *Cambarus* it is generally specialized as a local group oј setæ, or at most in *C. Clarkii*, as a less simple line.

The main features of the endopodite are, however, a shallow groove and a very stout ridge along the posterior face, external to the groove. This ridge ends distally as a swelling that is part of the specialized tip of the organ. More enlarged, Fig. 4, the tip shows the rounded head in which the ridge ends, the groove to the left of it and a depression to the right. The actual ending of the organ is a sort of complex edge, flattened from before back.

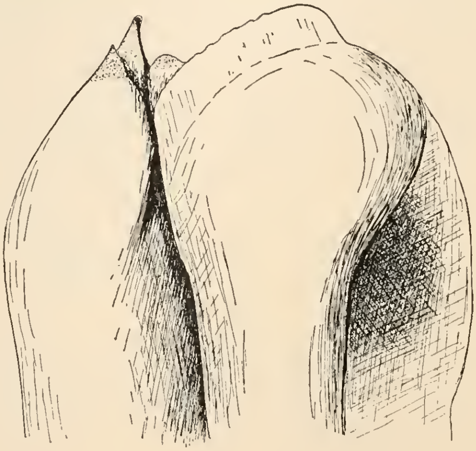

FIG. 4.

On the median side of the groove are two horny points, the shorter posterior, the longer anterior. The posterior is comparable to the large "spatula" and the anterior to the large "canula" of *Cambarus Montezumæ*. External to the groove is a short, blunt anterior point, comparable to the "ligula" of *C. Montezumæ* and a long thin knife edge that is a continuation oı the rounded head of the ridge. The organ appears fitted to open a slit into which sperm might flow from the groove.

In *C. Japonicus* the two first stylets lie side by side with divergent tips. In a male 45 mm. long, with stylets 8 mm. long, their tips were two millimeters apart, across the median line. Each stylet has the same structure as in *C. similis*, with only slight differences in proportion, but the groove opens more toward the median face and is not seen from behind, Fig. 5. While the tip Fig. 6 presents the same details as in *C. similis*, Fig. 4, the cutting edge is less sharply set off from the head of the ridge and runs out externally as a pronounced angle or spine, that is lacking in the former species.

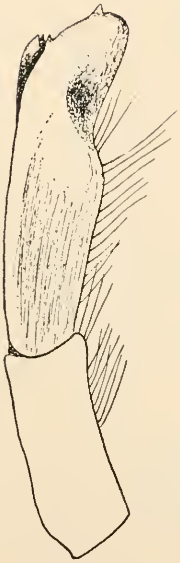

FIG. 5.

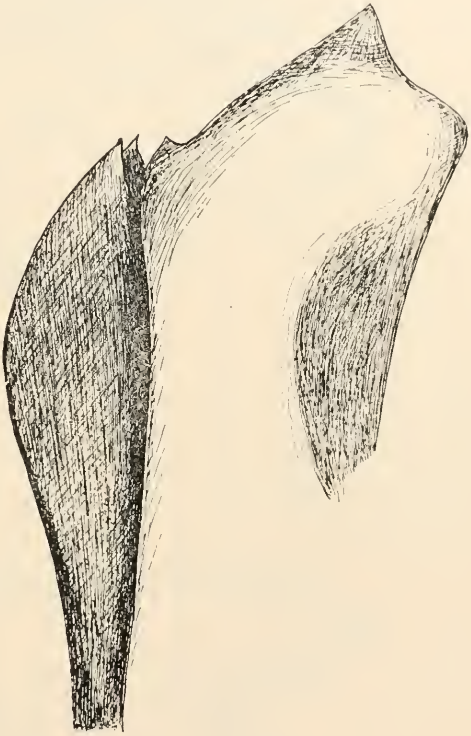

FIG. 6.

From an end view the tip of the organ of *C. Japonicus* is complexly modelled and suggest not a cutting tool but a probe to be forcefully inserted against resistance.

Serial sections of the first stylets of both species show the same inside structure. A thick, firm shell covers the soft areolar tissue and a delicate epidermis underlies the shell and is continuous with the areolar tissue. The internal anatomy and the external modelling is shown by the series of sections, Figs. 7, 8, 9, cut along the lines 7, 8, 9 of Fig. 3. The organ is essentially a thick, flat plate with a groove on its posterior face dividing it into a smaller median part that we will call the median mass and a larger external mass, *M.M.* and *Ex.m.* in Fig. 7.

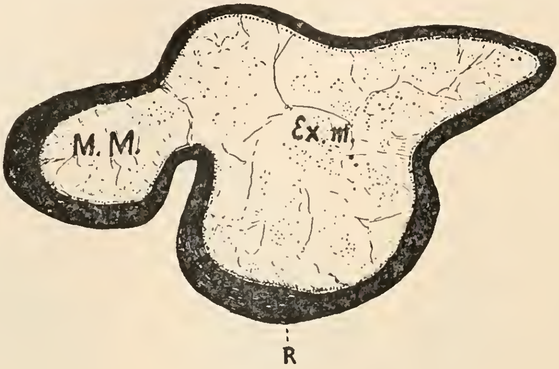

FIG. 7.

The groove is made much deeper by the fact that a great ridge, *R*, rises up from the external mass and forms the external boundary of the groove. In the middle of the course of the groove the ridge, Fig. 8, extends toward the middle line of the

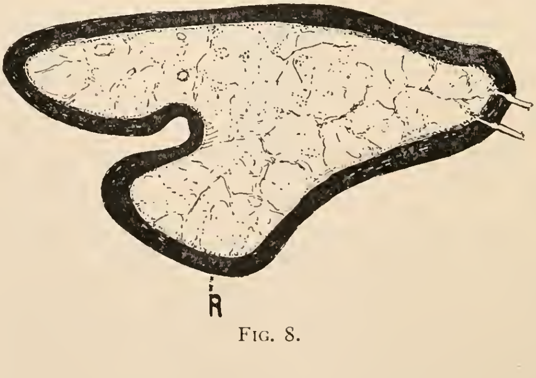

FIG. 8.

body parallel to the median mass so that the groove is here deep and narrow and opens out more to the median face of the organ. Toward the basal end, Fig. 9, the shallow groove is bounded by the diminishing terminal part of the ridge. At this level also may be seen the muscle mass that extends into the endopodite and indicates that the joint between the endopodite and exopodite may actually be used and the position of the tip of the organ be directed by this muscle. This muscle was also seen in cleared mounts in toto.

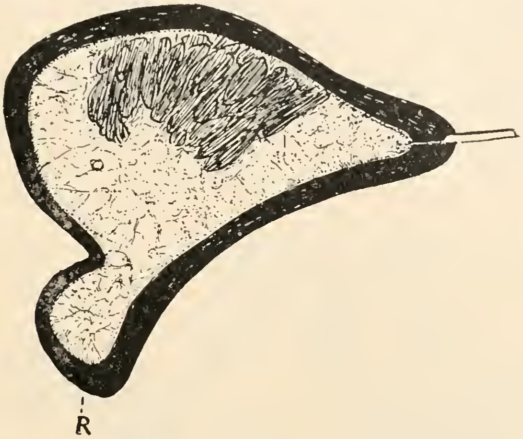

Fig. 9.

In the same manner in *C. Japonicus* sections show only a simple groove and large ridge, with the only difference that the groove faces more toward the median aspect of the organ, so that the above Figs. 7, 8 and 9 would well represent the condition in both species.

The second stylet preserves the usual pleopod form in that it is forked, or has both endopodite and exopodite. In *Astacus* (*Cambaroides*) *Japonicus*, Fig. 10, the exopodite is a slender obscurely segmented filament bearing few long setæ while the endopodite is the wide massive terminal part of the stylet. The tip of this endopodite, the flabellum, bears a long tuft of setæ and is evidently like the tip of the exopodite, but much enlarged. In the median edge of the endopodite there arises the extra element comparable to the "triangle" of *Cambarus*, that probably has some use in sperm transfer. This is a thick ridge that rises up as a free, thumb-like process directed diagonally across the endopodite. It has a marked angular elbow on the median side and terminates in an oblique and somewhat hollowed face posterior to the flabellum. This very simple representative of the triangle of *Cambarus* and the scroll of the American and European *Astacus*, bears still a few setæ, several upon the median and two or three upon the external border. In this it is intermediate between the above two crayfish.

The entire appendage seems crude and clumsy, either primitive or reduced.

This appears again in the other species of *Cambaroides*, *C. similis*, Fig. 11, which is like *Japonicus* but the setæ are very short and the flabellum more reduced. The dotted area in Fig. 11 is membranous. The posterior face is turned so that the

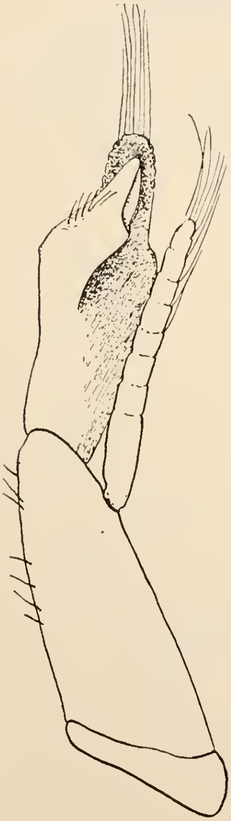

FIG. 10.

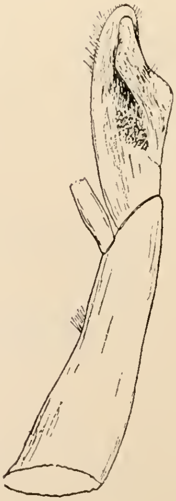

FIG. 11.

triangle can fit into the groove in the first stylet. In *Cambarus* the projection of the second stylet fits accurately to the groove of the first stylet and insures sperm transfer and in *Cambaroides* we can see that the projection upon the second will run in the groove of the first but it does not seem nicely adjusted to it.

Another departure from the finer adjustments of *Cambarus* may be inferred from the simpler mode of ending of the defferent duct. While in *Cambarus* it ends in a soft papilla that is fitted into the groove of the first stylet, in *Cambaroides* there is, at least in the preserved specimens, only a rounded, slightly raised area with a slit in it for the exit of the sperm.

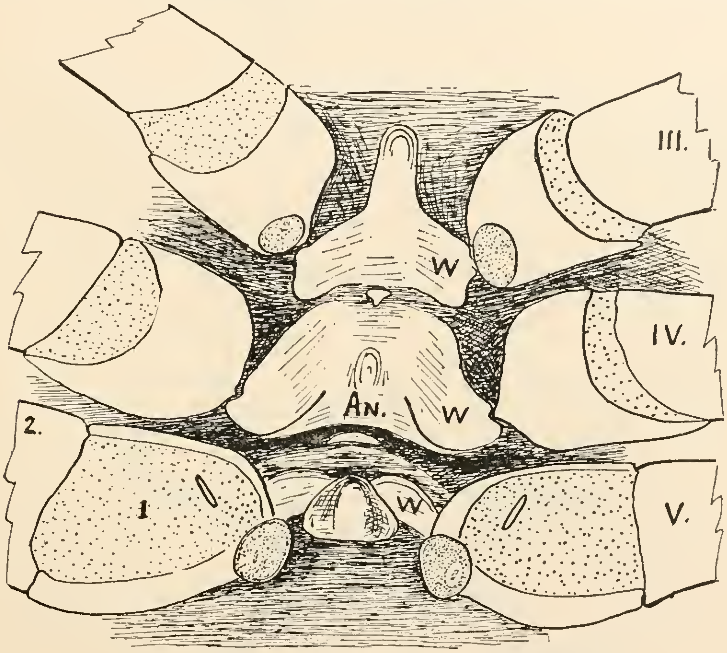

Fig. 12.

The under side of the thorax of the male, Fig. 12, shows the ending of the defferent duct as a small opening in a rounded raised area on the base of the fifth leg, right and left. In *Cambaroides Japonicus*, Fig. 12, these rounded areas are soft and the entire adjacent surface of the base of the leg is also membranous, as indicated by the dotted region, except for the minute hard oblique ridge isolated in the membranous area. In *Cambaroides similis*, the base of the fifth leg shows a large white area thought to be glandular, the area 1 in Fig. 13, and the ending of the defferent duct is in a solid projection with a slit-like orifice.

Whether in life a soft papilla can be projected from these orifices is doubtful, but the hard and slightly projecting areas can be put against the groove in the first stylet in such a way that we infer the sperm may be poured out into the groove of the stylet, with perhaps some aid from the second stylet.

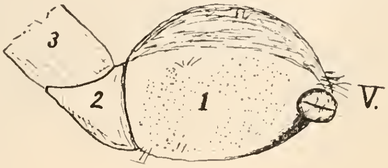

FIG. 13.

Turning now to the destination of the sperm transferred by the above male organs we fail to find upon the female any specialized receptacle. The under side of the thorax of the female *Cambaroides Japonicus*, Fig. 14, has a series of median plates between

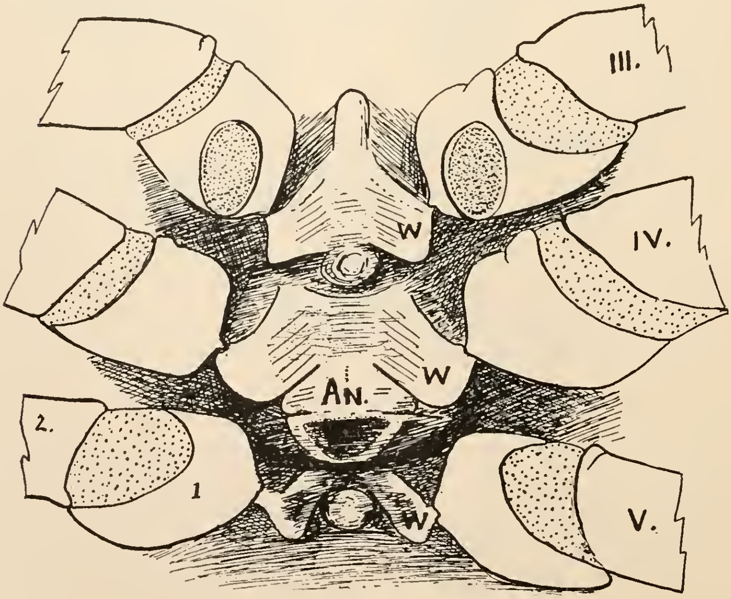

FIG. 14.

the limbs *III*, *IV* and *V*. Each plate expands toward the limbs as a lateral wing, *W*, each plate is also modified in its central part. Between the third limbs the central part shows a rounded boss and an anterior prolongation ; between the fifth limbs there is only a boss ; between the fourth limbs the median part of the plate is prolonged backward not as a boss but as a large expanse, the annular plate, *An*. This annular plate is subdivided into an anterior part, *An*, somewhat convex from side to side and a posterior part which is hollowed out as is poorly shown in Fig. 14.

The hollowed posterior part of the annular plate rises up dorsally at an angle with the horizontal anterior part.

The median depression is quite shallow and neither in surface views nor in sections is there any slit or internal pocket such as is characteristic of the annular plate of the genus *Cambarus*. Thus while in both *Cambarus* and *Cambaroides* there is an annular plate only in *Cambarus* is it provided with an internal cavity. It is the internal cavity in the annular plate that is filled with sperm. In these specimens of *Cambaroides* no sperm pocket is found.

In the male *Cambaroides*, Fig. 12, there is also an annular plate between the fourth limbs but it lacks the hollowed posterior part that is found in the female.[1]

In a specimen of *Cambaroides similis* the posterior part of the annular plate is less sharply hollowed out than in *C. Japonicus*.

The use of these various male and female parts can, as yet, only be inferred from comparisons with the organs of *Astacus* and of *Cambarus* whose use has been observed. But in applying the male to the female we are led to imagine that the stylets may deposit spermatophores between the fourth and fifth limbs.

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[1] In the individual male figured here there is an abnormal pair of structures that simulate the openings of oviducts. In the female the oviduct openings, Fig. 14, are large elliptical membranous areas upon the bases of the third limbs. In the male the openings of the defferent ducts are more elevated areas upon the bases of the fifth limbs, Fig. 13. There is thus both a difference in position and in character between the male and the female openings. The abnormal openings upon the third legs of the male figured in 12, are like female openings both in position and character though they are smaller than normal and the one upon the right of the animal is especially small (see also Fig. 2). This then seems to be a case of partial mixture of sex organs such as have been described in both *Astacus* and *Cambarus* (see *Am. Nat.*, 1909).

On the whole the sex organs of *Cambaroides* are more like those of *Cambarus* than like those of *Astacus*. That is, the hooks are present in *Cambarus* and *Cambaroides*, but not in *Astacus*. From their form we may infer that in *Cambaroides* they are used to hold the female just as they are used in *Cambarus*. The stylets in *Cambaroides* lack the flat scroll form of *Astacus* and are more like the stout complexly tipped organs of *Cambarus*, but they are much more simple. They doubtless serve to transfer the sperm as in both *Astacus* and *Cambarus*. In the female the annular plate of *Cambaroides* lacks the special sperm reservoir of *Cambarus* and is thus like *Astacus*, but it is more developed and somewhat hollowed out. In this respect it recalls the earliest phase of the ontogeny of the annulus of *Cambarus*.[1]

In brief the organs of *Cambaroides* are more simple than those of *Cambarus* but fashioned somewhat like them, suggesting some connection closer with *Cambarus* than with *Astacus*.

How are these facts to be interpreted? The general anatomy shows that *Cambarus* is the recent and *Astacus* the more unspecialized genus. Is *Cambaroides* a step from *Astacus* toward *Cambarus* or is it a step backward from *Cambarus ?*

Since *Cambaroides* has the same gill formula as *Astacus* and is like *Astacus* in having no sperm receptacle (as far as known), while on the other hand it has hooks like *Cambarus* and stylets similar to those of *Cambarus*, we may regard it as a genus separate from both *Astacus* and *Cambarus*.

It then becomes a question of the relative positions of these three genera. Granting that the larger number of gills is primitive and the small number derived we must assume either that the presence of hooks in *Cambarus* with few gills and in *Cambaroides* with more gills is a case of secondary convergence from parallel variation or else that it is a common inheritance. Ortmann has assumed the resemblances of *Cambarus* and *Cambaroides* due to convergence, but Faxon regarded them of more significance. The new facts as to the annular plate and the structure of the stylets will aid in the solution of this question ; with emphasis upon the sex organs as criteria of relationship, which has been the tendency of all recent work upon this group.

[1] Biol. Bull., X., Figs. 6, 7.

As elsewhere shown[1] some of the Penæidæ as well as the lobsters have sperm receptacles whence we may infer that a sperm receptacle was common to the ancestors of the crayfish. In such case the absence of sperm receptacle in *Astacus* would be due to loss, and the presence in *Cambarus* to retention of the ancestral mode of sperm transfer. If we suppose that the resemblances and differences of organisms are connected with chance variations it seems more likely that organs may have been independently lost, in separate animals, than that the same organ should have been independently acquired in separate organisms. We would then say that *Cambaroides* and *Cambarus* are closely related as both have retained the hooks and the general form of stylets of some ancestor but that *Astacus* and *Cambaroides* are not so closely related though both have independently lost the sperm receptacle while changing the form of stylets.

Adopting Ortmann's views as to the origin of the present distribution of crayfish we would modify it chiefly by the assumption of two migrations from Asia into America. We would think of ancestral Asiatic crayfish with many gills and a sperm receptacle of some sort filled by some use of the abdominal limbs. One set of descendants retaining more gills but losing the sperm receptacle became the *Astacus* of America and Europe as well as the crayfish of the southern hemisphere. While another set of descendants became *Cambarus* and *Cambaroides.* Part of this branch migrated into America and ultimately, in Mexico according to Ortmann's evidence, became the present *Cambarus* with reduced gills and highly specialized receptacle and stylets. The other part remaining in Asia independently lost the receptacle but retained the larger number of gills as well as the hooks.

We might then find in *Cambaroides* indications of the former presence of a sperm receptacle. As such we regard the hooks that have no known use except as aids for the filling of a receptacle. As such we regard the presence of ligula, spatula and canula at the tip of the stylet.

The absence of a tubule in the stylet, its clumsy form and the reduced prominence of its tip as well as the simplicity of the triangle of the second stylet might also be regarded as signs of degeneration.

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[1] *Zool. Anzeiger*, 1909.

But speculation is here very insecure and if use inheritance or some law of perfection were known it would be easy to argue that *Cambaroides* was an incipient *Cambarus* evolving from *Astacus.* In any case the common ancestor of *Cambarus* and *Cambaroides* must have been far back as it had the larger number of gills and as *Cambaroides* has the primitive characters of a well-developed flagellum on the first stylet and a muscle at the movable joint between protopodite and endopodite.

A diagram of the three genera would place *Astacus* and *Cambaroides* near together as having the same gill formula and as lacking a sperm receptacle, while *Cambarus* should stand apart as having a simplified gill formula and also very highly developed sperm-transfer organs, including a sperm receptacle. At the same time the diagram should indicate that *Cambarus* and *Cambaroides* kept together after departing from *Astacus*, and that later *Cambaroides* went off in the direction of *Astacus*, leaving *Cambarus* as at once the most specialized in its gills and the most conservative in its retention of the very ancient crustacean mode of sperm transfer by the employment of a sperm receptacle.

BALTIMORE, June 10, 1909.

STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN THE DOMESTIC FOWL.

III. A Case of Incomplete Hermaphroditism.[1]

RAYMOND PEARL AND MAYNIE R. CURTIS

Origin and General Character of Specimen.

From a chick hatched in the spring of 1907, at the Maine Agricultural Experiment Station, there developed the bird which forms the subject of this paper. This bird was a Barred Plymouth Rock and when adult presented externally the general appearance of a normal hen of this variety, so far as the characters body form and plumage color were concerned (cf. Plate I.). As the photograph in Plate I. shows, however, the head and neck resembled these parts in a cockerel. This resemblance was especially remarkable in respect to the size and shape of the comb and wattles. The comb was obviously much larger than the comb of a normal Barred Plymouth Rock hen and looked exactly like the comb of a male bird. This was also true of the wattles.

The dimensions[2] of the comb of this bird were as follows:

Length	88.4 mm.
Calculated height	25.1 mm.
Area	22.2 cm.^{2}

For normal adult Barred Plymouth Rock females the following average values for comb size have been found:[3]

Mean length	50.80 ± .56 mm.
‘‘ calculated height	10.57 ± .23 ‘‘
‘‘ area	5.59 ± .17 cm.^{2}

It is evident from these figures that the comb in this specimen greatly exceeds in size the average for females of the variety.

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[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station, No. 13.

[2] Made in accordance with the methods described by R. and M. D. Pearl in a paper ‘‘Data on Variation in the Comb of the Domestic Fowl,’’ *Biometrika*, Vol. VI., pp. 421–423.

[3] Pearl, R. and M. D., *loc. cit.*, p. 427.

In regard to behavior this bird resembled a normal hen rather more than a cock. She was never heard to cluck, however, or to make any of the sounds which normal active hens make in the course of the day's work. This bird probably never laid an egg, though we are unfortunately not able to make an absolute statement on this point. The egg records of the station show an egg to the credit of this bird on November 7, 1907. This was the only egg ever recorded for this bird, and it is undoubtedly an erroneous record. As will presently appear, the condition of the sexual organs was not such as to indicate that they had ever been functional.

Cockerels placed in the pen with this bird would try to fight with her as if she were a cockerel; but she would not fight. We have no evidence that a cockerel ever attempted copulation with this bird. These facts are of interest in relation to the question of the basis of sex-recognition and the assortative mating known to occur among fowls. Is a normal pullet with an unusually large comb less likely to have her eggs fertilized than a bird with a smaller comb?

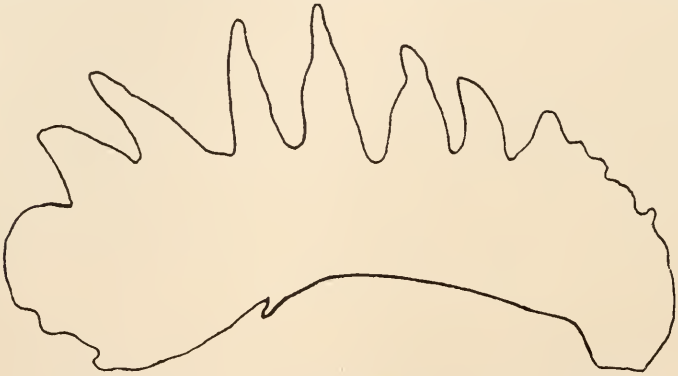

Fig. 1. Outline of the lateral aspect of the comb of the Barred Plymouth Rock hen described in this paper. This outline is actual size.

This bird was observed occasionally to take the position of a cockerel about to crow and attempted to crow but never succeeded in very closely approximating the sound of a normal cock bird. The bird was never seen to attempt to tread a hen.

AUTOPSY.

The appearance and behavior of this bird led to the suspicion that it represented a case of true hermaphroditism. On August 24, 1908, the bird was killed and a post mortem examination made. The weight of the body after bleeding was 2,725 grams. The body cavity contained much fat. The alimentary tract and attached viscera were entirely normal. The following measurements were made:

From gizzard to origin of cœca	167 cm.
Longest cœcum	22 "
From origin of cœca to cloaca	13 "

The following weights were taken:

Gizzard	125 gms.
Liver	44 "
Heart	9.5 "
Spleen	4.5 "

On the left side of the body was a normal oviduct. There was an ovary in the usual position. It was of about the size of the ovary of a laying hen after the removal of large yolks. It had a coarsely granular appearance and showed many folds. There were no eggs visible and its surface did not have the ragged appearance, due to ruptured follicles, which is characteristic of the ovaries of laying hens. The rest of the urinogenital system was completely covered by fat. The part of the body containing this organ system was hardened in formalin for further dissection.

GROSS ANATOMY OF REPRODUCTIVE ORGANS.

Dissection confirmed the suspicion of hermaphroditism. On the left side were the female-like reproductive organs described above while on the right side there was a set of organs similar to those of the normal male. The gross anatomy of the reproductive system in this bird is shown in Plate II., Fig. 1.

The female organs were more nearly normal than the male. The ovary, like a normal ovary, was ventral to the craniad lobe of the left kidney, covering, when viewed from the ventral side, all but the caudo-lateral angle of this lobe. It extended past the cranial margin of the kidney in the hermaphrodite nearly to, and in the normal hen with which comparisons were made, slightly beyond the fourth rib. In the hermaphrodite the ovary was developed less on the medial side than in the normal hen. The measurements of greatest length and breadth of ovary in the hermaphrodite were 29 mm. by 16 mm. while in the normal hen they were, excluding the projecting yolks, 34 mm. by 20 mm. The ovary was attached to the body wall near the middle line by a thick stalk-like portion. This appeared perfectly normal. Its longest dimension (cranio-caudal) was 15 mm. compared to 18 mm. in the normal bird. The external appearance of the ovary was quite different from that of a normal ovary. It seemed to be a coarsely granular but otherwise homogeneous mass covered by peritoneum and minutely and very irregularly folded. It did not have the ragged appearance of the normal ovary and the minute folded masses did not look like the yolks of similar size in the normal ovary. They seemed to be folds on the surface of a homogeneous mass rather than small spheres of yolk enclosed in follicles.

The oviduct was normal in appearance and in position. The mouth of the funnel faced the ovary while the cranial ends of the lips were fused and extended across the left kidney to the fourth thoracic rib some distance laterad of the cranial end of the ovary. The caudal ends of the lips also fused and were attached to the ventio-median margin of the ligament which holds the convolutions of the oviduct in place. The oviduct presented the same principal convolutions as a normal active oviduct. It was larger than the oviduct of the adult hens we have examined which had never laid and the ovaries of which did not show a number of yolks. Table I. gives the lengths of the oviducts we have been able to examine in this condition.

Table I.

Dimensions of Oviducts of Pullets which have Never Laid and which have no Growing Yolks on Ovary. Hermaphrodite Included for Comparison.

Band Number of Bird.	Length of Oviduct in cm.	No. of Yolks Above 1 cm. on Ovary.	Total Number of Eggs Laid.
Hermaphrodite 16 D	47.0	0	0
129 E	13.0	0	0
297 E	20.0	0	0
222 E	23.5	0	0

The length of the oviduct of the hermaphrodite hen 16 as given in the first line of this table is 47.0 cm. This is just twice the length of the next shorter oviduct, that of hen 222 E. The oviduct is however smaller than in hens actively engaged in egg production in the middle of a laying period. It compares most nearly in size with the oviduct in hens which have ceased laying from 5 to 16 days before examination, or to those with 4 to 6 large yolks on the ovary. In order that this comparison may be readily made Table II. is introduced. This table is compiled from records in the archives of the laboratory and gives certain data in regard to normal hens having oviducts between 44.5 cm. and 50.5 cm. long, *i. e.*, of approximately the same length as that of the hermaphrodite hen No. 16. The table includes the following data: (*a*) length of oviduct in cm.; (*b*) the number of yolks 1 cm. or more in diameter on the ovary at the time of autopsy; (*c*) the number of days elapsed since the last egg was laid. In case the bird never laid the sign ∞ is used to denote this fact.

TABLE II.

Data from Normal Hens on Oviducts of Same Size as that of Hermaphrodite Bird.

Band Number of Bird.	Laboratory Autopsy Number.	Length of Oviduct in cm.	Number of Eggs 1 cm. or More in Diameter on Ovary.	Number of Days Since Last Egg Was Laid
Hermaphrodite 16	175	47.0	0	∞ (?)
260 D	117	47.0	0	8
15 D	124	50.0	0	5
213 D	133	46.0	0	7
788 D	137	45.0	0	9
38 D	151	46.0	0	8
405 D	171	50.0	4	9
317 E	179	50.0	4	∞
480 E	202	49.0	2 absorbing	16
314 E	236	48.5	2 "	10
96 E	237	49.0	6 "	∞
429 E	248	48.5	14 (hard, absorbing)	9

From the table it is apparent that the oviduct in the hermaphrodite hen was in essentially the same condition as that of a normal hen which has recently completed an egg-laying period (clutch).

Internally the oviduct of the hermaphrodite was differentiated into the regions characteristic of the normal oviduct. The funnel walls were thin and transparent. Grandular ridges appeared at the end of the funnel and became gradually heavier and higher. In the albumen-secreting portion of the tube they were heavy, high and irregularly lobed. The ridges at the cranial end of the isthmus were thin and straight but did not preserve that character so strictly as in a normal oviduct, so that near the shell gland they resembled the ridges in the albumen-secreting portion. The shell gland ridges were high, very irregular and much lobed. These turned dark in the preserving fluid as we have often noticed to be the case with normal oviducts. The vagina had the characteristic low straight ridges. The dimensions of the various parts of the oviduct were as follows :

Length of funnel neck	2.5	cm.
Width of flattened tube at point where funnel passes into albumen portion	0.6	"
Length of albumen portion	18.5	"
Width of flattened tube at widest part of albumen portion	0.9	"
Length of isthmus	7.0	"
Length of shell gland	7.5	"
Width of widest part of shell gland	2.0	"
Length of vagina	11.5	"

The opening of the oviduct was in the normal position, slightly to the left of the midventral line. The margin of the opening was folded, but was inconspicuous while in a laying hen it protrudes a little into the cloaca. A large probe was passed from the vagina into the cloaca demonstrating a natural opening between these organs.

The left suprarenal body was covered by the cranial end of the ovary as in normal cases.

Directly opposite the middle of the ovary on the left side of the body was a small irregular, though generally ovoid organ, the testis (Plate II., *T*). This organ was 9 mm. in length by 6 mm. in greatest breadth. It was attached to the body wall by its broad side with the more convex side median and the nearly straight side lateral. This organ did not appear macroscopically like a testis but looked to the naked eye or through the hand lens, like a small mass of the same sort of tissue as the ovary already described, but covered with an additional layer of connective tissue which obscured the minute foldings.

From the lateral side of the testis a duct passed to the cloaca running parallel to the median line and ventral and lateral to the ureter. This tube was nearly straight throughout its course, but had a few convolutions near the cloacal end. It had the position and appearance of a normal vas deferens in a young cockerel. The tube was heavy walled and gradually increased in diameter caudad. Sections showed that this duct had a definite lumen. There was no enlargement comparable to a seminal vesicle. It was not possible to demonstrate an opening into the cloaca.

Histology of the Left Genital Gland (Ovary).

The left genital gland was much less finely lobulated than a normal ovary. The large lobules had smooth contours. The organ was covered with a layer of peritoneum. Over most of the surface the cells of this layer were nearly cubical but in some portions they were shorter than broad while in other regions they were nearly twice as tall as broad. Over a few small areas there was an outward proliferation of this epithelium so that evaginated folds of the epithelium four to six cells deep projected from the surface. In a few cases these evaginated ridges were still further folded along their lateral margins.

Beneath the peritoneal layer was the tissue which formed the bulk of the organ. This was a highly cellular but much vacuolated tissue, the cells of which were not unlike the cells of the stroma of a young ovary. This tissue was nearly uniform throughout the organ. In the vacuoles of this tissue were found, in many portions of the organ, irregular non-cellular masses which stained deeply with acid stains, especially eosin. Some of these masses were surrounded by a single layer of very much flattened cells. They did not appear like ova nor did the surrounding cells resemble normal follicle cells. In the part of the organ ventral to the suprarenal body were a few spherical portions of the stroma-like tissue which were more dense and took a deeper stain with hæmatoxylin. These portions did not differ in other particulars from the surrounding tissue.

The stroma-like tissue contained few blood vessels but a highly vascular connective tissue penetrated the organ from the stalk. This tissue appeared like a core to the organ projecting into the larger lobules in tongue-shaped masses.

No Graaffian follicles or Pflüger's tubes were found, though series of sections from all parts of the gonad were examined. The general histological structure of the organ was such as to indicate that it was in a degenerating condition at the time the bird was killed. This process of degeneration had gone so far that nothing like normal ovarian tissue was to be found. Whether at any time in the life of the bird any part or all of the ovary had been normal in structure, it is impossible now to say. The condition of the gland at death afforded no certain evidence either for or against this view. That oögenesis, however, could not have gone beyond early stages during the later life of the bird is made probable by the fact that it did not lay (except for the single doubtful egg noted on p. 272), although possessed of a normal oviduct.

The net result of the microscopical examination of the left genital gland, which had the normal anatomical relations of an ovary, is negative.

Histology of the Right Genital Gland (Testis).

The limiting membrane of the right genital gland was not very thick and was poorly preserved in our sections. Such parts of it as were intact seemed to have a cellular outer layer with a fibrous inner layer. We could not be sure of futher histological details nor could we determine the extent of this tunic.

The gland contained no normal seminiferous tubules but showed evidence of tubular origin. The central portion was more dense than the periphery and in this more dense portion a few places showed the cells arranged as if small cellular rods had been cut in various planes. These rods might be considered tubes without lumen. They were formed by a single layer of nearly cubical cells, about the size and form of the epithelial cells of the seminiferous tubules at the age when these form a single layer nearly filling the lumen. Around these rods was a thin layer of fibrous tissue. Between the dense central portion and the periphery the epithelial cells gradually disappeared so that the greater portion of the gland appeared to be a connective tissue skeleton representing the basal membranes of the tubes, and the intertubular stroma of the young gland. Most of the tubes formed by the remaining basal membranes contained a few cells but these were irregular and had lost their epithelial character. In some of the tubes were eosin-staining non-cellular masses like those found in the left genital gland. In the right gland we did not find cells surrounding these masses.

On the dorsal side of the organ was a mass of tubules. Those seen in each section varied considerably in diameter but they were much smaller toward the cranial end of the organ. This mass of tubules extended the full length of the testis. The tubes were lined with simple, heavily ciliated, columnar epithelium. Outside this, especially in the larger tubules, could be distinguished one or more layers of smooth circular muscle cells. The tubes were imbedded in connective tissue. This tubular structure was in all essential particulars precisely like a normal epididymis and without any question represents that organ. A photograph of a section through the epididymis is shown in Plate II., Fig. 2. The magnification used in this figure is low, but on the original negative the cilia on the cells lining the tubes can be plainly seen.

On the median side of the testis and lying for the most part at the side of the epididymis, though in some portions extending between the gland and the epididymis, was a mass of very vascular connective tissue.

Sections of the vas deferens at about the middle of its length showed it to be a tube considerably larger in diameter than the largest part of the epididymis. It was lined with columnar epithelium showing, in some sections, two rows of nuclei close together. In some sections cilia could be distinguished, but they were not so easily demonstrated as in the epididymis. There was a subepithelial layer of non-muscular tissue, probably the mucosa, and outside this a thick layer containing smooth muscle fibers. We were unable to distinguish different muscular layers in our sections.

The lumen of the vas deferens and also parts of the epididymis contained masses which stained strongly with eosin. These masses included irregular fragments that took the chromatin stain.

In general, the histological study of the right genital gland led to the same conclusion as did that of the left, namely, that we were dealing with a degenerating structure. As is indicated in the foregoing description, however, the right gland approached somewhat more nearly to the normal than did the left. Whether this organ was even functional, however, in the sense of containing actively dividing spermatogonial cells, cannot be determined from the evidence afforded by the histology of the gland at death. It may or may not have been. One cannot tell. So then again our results on the question as to whether actual spermatogenesis occurred in this hermaphrodite fowl are negative. All that can be positively affirmed is that at the time when the bird was killed, both sexual glands were in an inactive and degenerating condition.

Discussion of Results.

The case above described presents a number of points of considerable theoretical interest which we may now, with the facts in hand, proceed briefly to discuss.

The first point to which we would direct attention is the peculiar combination or correlation of sexual characteristics (primary and secondary) which existed in this bird. Externally it presented a condition essentially similar to the rarely observed antero-posterior gynandromorphism of insects. Anteriorly the bird was male in its external somatic characters; posteriorly it was female. The truth of this statement may be demonstrated in a striking manner by placing the edge of an opaque card along a line connecting the letters *a* and *b* in Fig. 1 of Plate I. and turning the card about this edge as an axis so as to expose alternately the anterior and posterior parts of the bird. When the card covers the posterior part of the bird what one can see (*i. e.*, the anterior part) is unmistakably and indubitably male. On the contrary, when the anterior part is covered by the card, what of the bird is then to be seen is equally unmistakably female. The "maleness" and "femaleness" of these two portions of the body extend to the most minute details of structure, perhaps not apparent to anyone not perfectly familiar through first-hand practical experience with poultry and particularly Barred Plymouth Rocks. Thus the beak — which is not ordinarily reckoned as a secondary sexual character — in this bird is to the fancier unmistakably that of a male.

It is certainly a remarkable fact that with this perfectly clear and definite somatic gynandromorphism there is associated an absolutely inactive condition of the primary sexual organs, so far as the functions of spermatogenesis and oögenesis are concerned. The case shows clearly enough that the secondary sexual characters of both sexes *may* exist without the accompaniment of functionating germinal epithelium in the same individual. It does *not* prove that the secondary characters may originally *develop* in the absence of the functioning of the primary glands, because of the uncertainty as to whether either of the glands was ever functional in this specimen.

There has accumulated in recent years a considerable mass of evidence,[1] particularly from medical, surgical and gynecological workers, tending to show that the development of secondary sexual characters is in some way controlled through internal secretions (containing hormones) produced in some part or parts of the primary sexual apparatus. While the general fact of such a relationship is now to be regarded as quite definitely established, the details of the process are as yet by no means worked out. Whether these secretions are elaborated in cells of the germinal epithelium proper, from interstitial or stromal cells, or from the accessory parts of the reproductive apparatus (*e. g.*, epididymis, oviduct, etc.) is, in general, still unknown. It might at first thought be supposed that the present case, inasmuch as the glands are degenerate and non-functional whereas the accessory male and female organs (epididymis, vas and oviduct) are complete and normal, afforded evidence in favor of the view that these latter organs are sources of internal secretions influencing secondary sexual characters. Any presumptive warrant for such an inference, however, is largely if not entirely taken away by evidence of another kind. We have conclusively shown, for example, in unpublished experimental work that complete or partial removal or ligation or section of the oviduct in the domestic fowl, undertaken before or after the oviduct has become functional, is without any effect whatever on the development or persistence of the female secondary sexual characters. The fact that in man vasectomy (practised, for example, in Indiana for the sterilization of criminals and certain other undesirable citizens) produces no effect whatever on secondary sexual characters or the sexual appetite is again evidence in the same direction.

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[1] It seems unnecessary to print *in extenso* here the long list of literature which exists on this subject. An introduction to this literature will be found in Morgan's "Experimental Zoölogy," Chapters 28 and 29, and in Bayliss, W. M., and Starling, E. H., "Die chemische Koordination der Funktionen des Körpers," *Ergeb. der Physiol.*, Jahrg. V., pp. 664–697, 1906.

The present case, of course, affords no direct evidence as to whether a secretion influencing secondary sexual characters may not be produced by the interstitial or stromal cells.

A further point of considerable interest lies in the fact that in this bird we have a fully developed, normal, and so far as can be told, entirely functional oviduct in the absence of a functional ovary. Normally in the hen the oviduct is in an atrophied, non-functional condition at times when laying is not going on, *i. e.*, when the ovary is not functioning. In the young pullet the oviduct stays in an infantile condition until the oöcytes begin to enlarge by the deposition of yolk just before laying begins. As the yolks approach the size at which they are separated from the ovary the albumen-secreting and other glands of the oviduct become enormously enlarged and the whole organ passes into the "laying condition." After laying stops the glands quickly atrophy and the whole organ goes back to the adolescent condition. In other words, there is in the normal bird a close correlation between the functioning of ovary and oviduct. There is, of course, a similar apparent correlation between ovary and uterus in mammals.[1] Now in this hermaphrodite specimen the correlation is apparently upset. We have the oviduct in "laying condition" in a bird in which the ovary is absolutely non-functional so far as ovulation is concerned. The two cases of hermaphroditism in the domestic fowl described by Shattock and Seligmann[2] essentially parallel ours in this regard. In both cases they found a well-developed oviduct, though the ovary was distinctly not in *ovulating* condition.[1] These cases point strongly to the idea that the mutual interrelationship between ovary and oviduct in birds is very far from being of such a simple character as one would be led to infer from observation of normal specimens. Here, as in other instances, teratology may furnish the clue for the elucidation of a normal physiological process.

---

[1] Here the brilliant work of Dr. Leo Loeb is establishing, by means of analytical experimentation, the causal factors in the physiology of the uterus. Cf. Loeb, L., "The Production of Deciduomata and the Relation between the Ovaries and the Formation of the Decidua," *Jour. Amer. Med. Assoc.*, Vol. L., pp. 1897–1901. June 6, 1908.

[2] Shattock, S. G., and Seligmann, C. G., "An Example of True Hermaphroditism in the Domestic Fowl, with Remarks on the Phenomenon of Allopteratism," *Trans. Pathol. Soc. London*, Vol. 57, pp. 69–109, Plate I., 1906. "An Example of Incomplete Glandular Hermaphroditism in the Domestic Fowl," *Proc. Roy. Soc. Medicine*, Vol. I., pp. 3–7, 1907.

Summary.

The purpose of this paper is to describe in detail a case of incomplete hermaphroditism in the domestic fowl. It is shown that:

1. In its external somatic characters the specimen was an antero-posterior gynandromorph.

2. Internally the bird possessed on the left side a large, lobulated gland in the position and anatomical relations normal to the ovary. There was also a fully developed, normal oviduct, in functional condition on the left side of the body.

3. On the right side of the body was a small organ in the position and anatomical relations normal to the right testis. Attached to this organ was a normal epididymis and vas deferens leading to the cloaca.

4. Microscopical examination showed that both sex glands were in a condition of extreme degeneration. Neither spermatogenesis or oögenesis could be found in any part of either gland.

5. Certain theoretical aspects of the case are discussed.

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[1] These authors did, in these cases, succeed in finding some evidence of actual oögenesis, but not of ovulation, either past or prospective.

Explanation of Plate I.

Showing the hermaphrodite specimen described in the text. A normal male and female of the Barred Plymouth Rock breed are shown for comparison.

Fig. 1. Hermaphrodite specimen. A line connecting the letters *a* and *b* marks the division region between the male and female portions of the gynandromorphic condition. Cf. text, p. 280.

Fig. 2. Normal Barred Plymouth Rock cockerel.

Fig. 3. Normal Barred Plymouth Rock pullet.

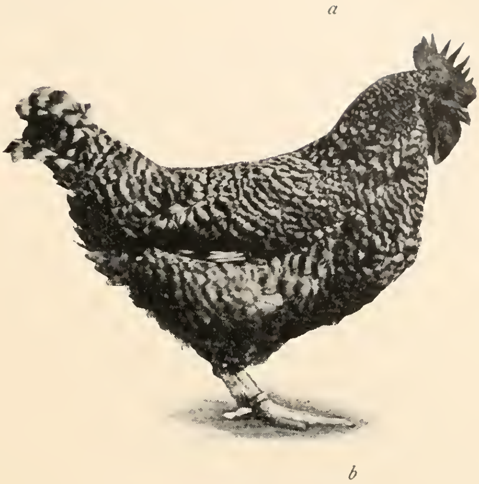

FIG I

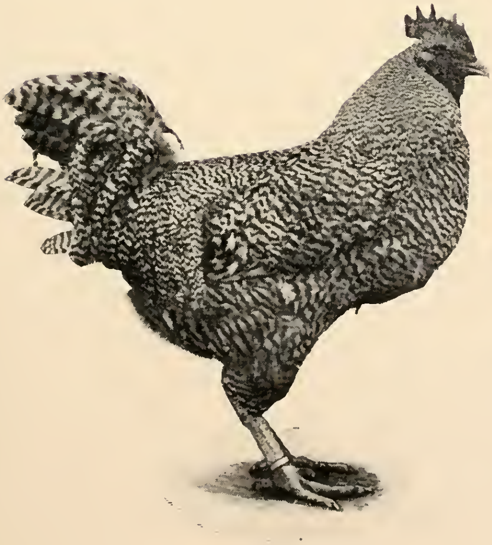

FIG. 2.

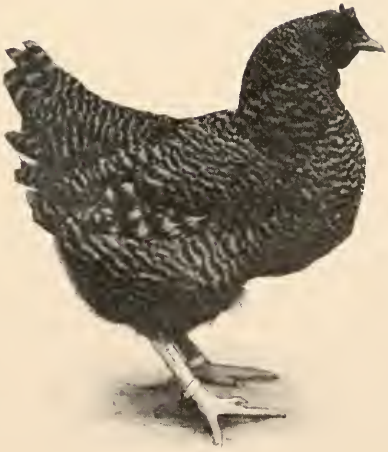

FIG. 3.

Explanation of Plate II.

Fig. 1. Photograph showing the gross anatomy of the genital organ of the hermaphrodite specimen. *O*, ovary. *F*, funnel mouth of oviduct (ostium tubæ abdominale). *S*, region of shell gland of oviduct. *T*, testis. *V.D.*, vas deferens. *U*, right ureter. A black card is placed behind the vas deferens and ureter in the lower portion.

Fig. 2. Microphotograph of section through epididymis. Obj.: Spencer 32 mm.; 6 × compensating ocular.

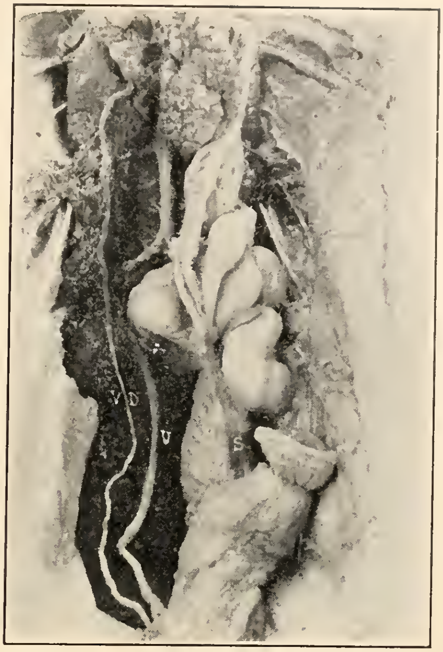

FIG. 1.

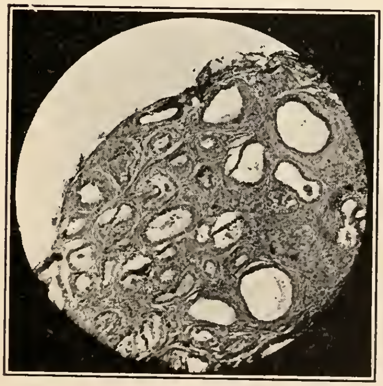

FIG. 2.

FURTHER STUDIES ON THE LIFE CYCLE OF PARAMECIUM.

LORANDE LOSS WOODRUFF.

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I. Introduction.

The life cycle of infusoria has been the subject of numerous investigations since Ehrenberg suggested on *a priori* grounds that the protozoa are so simply organized that they are not subject to natural death, and Dujardin opposed the view and maintained that the life history of infusoria comprises a cyclical change in vitality which terminates in death.

Bütschli ('76), Engelmann ('76), Maupas ('88; '89), Joukowsky ('98), Simpson ('01), Calkins ('02; '04), Woodruff ('05), Popoff ('07) and Gregory ('09) have all advanced evidence tending to show that infusoria when bred under somewhat constant culture conditions pass through a more or less definite physiological cycle. This cycle is characterized by an initial high potential of division which gradually is expended until reproduction finally ceases, and death puts an end to the cycle unless conjugation is permitted or artificial stimuli are employed. Characteristic morphological changes, both cytoplasmic and nuclear, appear in many cases as "senile degeneration" increases.

Enriques ('08) in a recent paper has again opposed the idea of old age and physiological death in protozoa and has contended that the results which support the cyclical character of the infusorian life history have been obtained by faulty culture methods. The conclusion of Enriques is, I believe, somewhat too sweeping, and is based in part on a misunderstanding of the methods by which the most extensive cultures have been conducted. The work on the infusorian life history has clearly shown that many species of infusoria, when bred on a more or less constant culture medium, pass through quite definite cycles. Calkins, Woodruff, and Gregory have shown also that specific changes in the environment at critical times may "rejuvenate" a culture and lengthen its life for long periods. It is demonstrated, I believe, that the life history of the infusorian is cyclical when subjected to a constant environment, and it is also demonstrated that the life history may be lengthened by the timely use of various stimuli.

I have defined a cycle as "a periodic rise and fall in the fission rate, extending over a varying number of rhythms, and ending in the extinction of the race, unless it is 'rejuvenated' by conjugation or *changed environment*."[1] This suggests the idea that it may be possible to eliminate the cyclical character of the division rate by *constantly* subjecting the organisms to a varied environment and the present investigation is devoted to this aspect of the problem. In a former paper[2] I have given an outline of my studies up to May, 1908, on the life history of *Paramecium* when subjected to a varied environment. The present paper presents the data to June 29, 1909.

II. Methods.

A "wild" *Paramecium aurelia* (*caudatum*) was isolated from a laboratory aquarium on May 1, 1907, and placed in about five drops of culture medium on an ordinary glass slide having a central ground concavity. When this organism had divided twice, producing four individuals, each of these were isolated on separate slides to start the four lines, *I–a*, *I–b*, *I–c* and *I–d* which compose this culture (Paramecium I).[3] The culture has been continued by the isolation of an individual from each of these lines almost daily throughout the life of the culture up to the present time (June 29, 1909). A record has been kept of the daily divisions of each line, and the average rate of division of the four lines of the culture and this again averaged for five- ten- and thirty-day periods has been plotted (cf. Figs. 1, 2 and 3). Permanent preparations have been preserved at various periods in the life history for the purpose of studying the cytoplasmic and nuclear changes, if present.

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[1] Woodruff ('05).

[2] Woodruff ('08²).

[3] For further details in regard to technique see Woodruff ('05).

The culture was carried on at the Thompson Biological Laboratory of Williams College, Williamstown, Mass., during May and June, 1907; at the Marine Biological Laboratory, Woods Holl, Mass., during July and August, 1907 and 1908; and at the Sheffield Biological Laboratory of Yale University, New Haven, Conn., from September, 1907, to July, 1908, and from September, 1908, to the present time (June, 1909).

During the first nine months of the work the culture medium was made of hay or grass; but, except during certain periods in which the culture was employed as a control for special experiments,[1] the infusion was made with hay from various localities, and different proportions of hay and water were used almost daily. Water from different sources was employed. The temperature of the infusion was always raised to the boiling point. In some cases the infusion was used as soon as it had again attained the room temperature; in others, it was allowed to stand for twenty-four hours before it was employed.

From February, 1908, to the present time, June, 1909, however, a more varied culture medium was employed. *Paramecium* will thrive in nearly any infusion which may be made from materials collected in ponds and swamps, and therefore, in an endeavor to supply as far as possible all the elements which may be encountered in the usual habitat of the organism, water was taken from ponds, laboratory aquaria, etc., together with their animal and plant life. In other words, no definite method was used in selecting the material, but it was simply collected at random from what might be the abode of infusoria, and thoroughly boiled. Probably the only condition present in the life of this culture which could not be encountered by a wild *Paramecium* was that the water had been boiled, but this was essential in the experiments in order to obviate the possibility of the contamination of the culture by an active or encysted wild specimen. Conjugation was impossible in the direct lines of the culture on account of the frequent isolations and change of medium.

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[1] Woodruff ('08[1]).

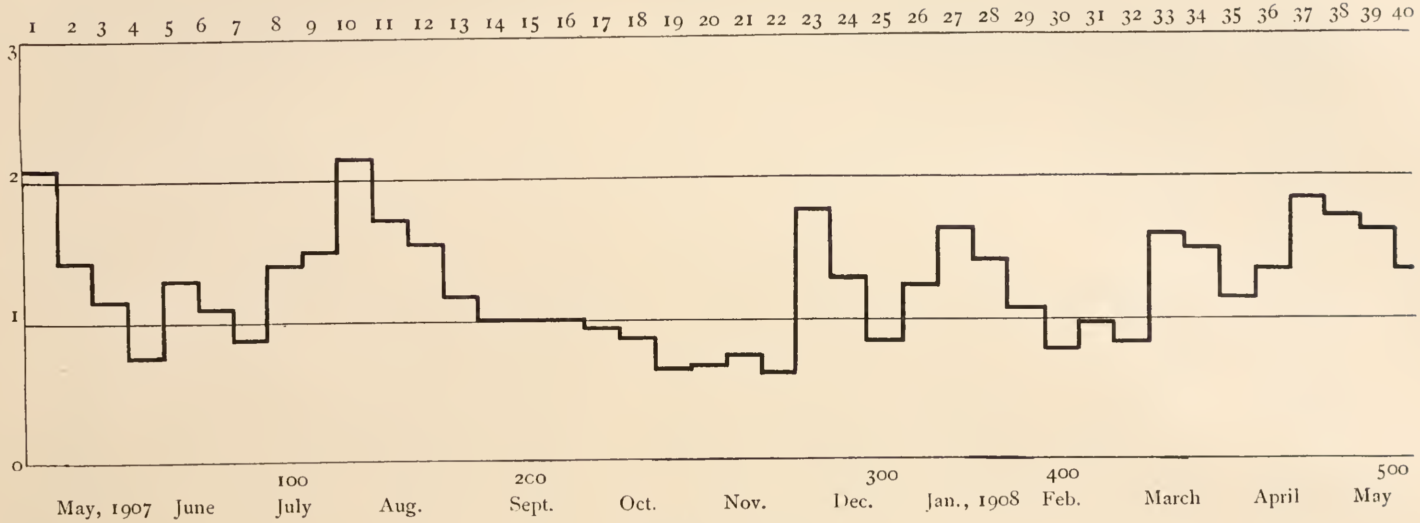

DIAGRAM I. PART I.

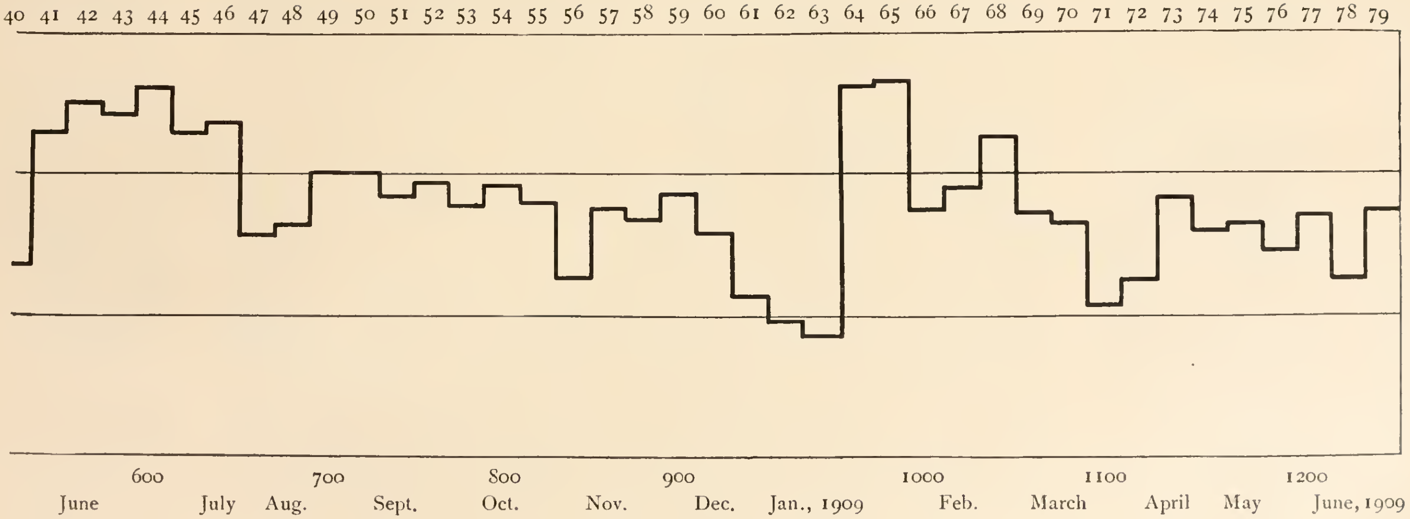

DIAGRAM I. PART 2.

FIG. 1. (Ten-day periods.) Complete history of *Paramecium aurelia* (*caudatum*), Culture I, from start on May 1, 1907, to the present time, June 29, 1909, at the 1,238th generation. The rate of division is averaged for ten-day periods. The ordinates represent the average daily rate of division of the four lines of the culture. Above, the numbers of the ten day periods are indicated. Below, are designated the periods in which the fifteenth day of the respective months fell. The figures, 100, 200, etc., represent generations and are placed in the periods in which they were attained.

III. Description of Cultures.

Culture I has attained, during the first twenty-six months of its life, the 1,238th generation. The average rate of reproduction for the entire period has been over one and a half divisions per day, and during not a single ten-day period has the average rate fallen as low as one division in two days, while during several ten-day periods the rate has averaged over two and a half divisions per day.

Fig. 1 shows, by the familiar block method, the average rate of division of Culture I for ten-day periods. Especial emphasis is put on the character of this curve as the results of Calkins' cultures are plotted for ten-day periods. A study of this diagram shows that the life history falls naturally into two parts. The first extends from period one to period thirty, and the second extends from period thirty to the present time. These two major parts of the curve are coextensive with different methods in the treatment of the culture. The culture medium used during the periods covered by part one was very much more uniform than that used during part two; the decidedly varied environment not being employed until February, 1908. The effect of this treatment is shown in the decided change in the character of the curve of the division rate from that period to the present. The general vitality of the protoplasm is considerably higher as is shown by the fact that only once since that time has the curve for a ten-day period fallen below one division per day, and that period represents the culmination of the more or less severe treatment which the culture received when it was carried to Baltimore during convocation week. However, to the change of water and other conditions incident to the journey is apparently to be attributed the stimulus which enabled the culture to attain a short time after an average rate of nearly two and three quarters divisions per day (see Fig. 1, periods 64 and 65), the highest reproductive power shown in the life history for any ten-day period.

A study of Fig. 2, which is plotted by the same method as Fig. 1 except that the periods are of thirty-day instead of ten-day duration, also shows clearly the effect of the varied culture medium which has been in use from February, 1908. The rate

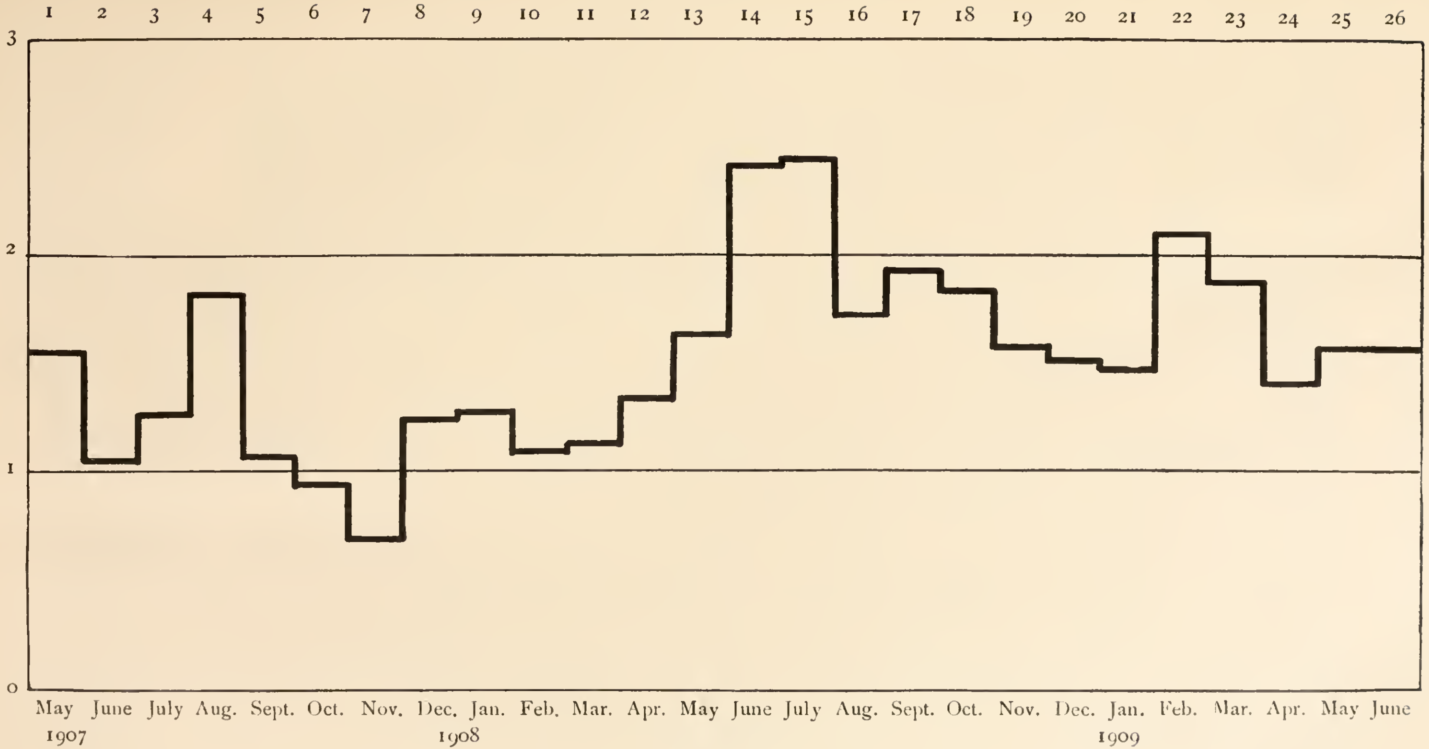

DIAGRAM II.

FIG. 2. (Thirty-day periods.) Complete history of *Paramecium aurelia* (*caudatum*), Culture I, from start on May 1, 1907, to the present time, June 29, 1909, at the 1,238th generation. The rate of division is averaged for each month of the life of the culture. The ordinates represent the average daily rate of division of the four lines of the culture.

of reproduction has never again fallen as low as it was when this treatment was begun. When averaged for thirty-day periods the highest rate of division appears in June and July, 1908; the great rise in vitality which occurred in February, 1909, and is shown in periods 64 and 65 of Fig. 1 (ten-day periods) is not so conspicuous, as the average is reduced by the low rate of fission during the two periods preceding.

A similar examination of Fig. 3, in which the rate of division is averaged for five-day periods, is not so instructive because the influence of the rhythms is more clearly brought out during short periods, so that the general trend of the curve of the life history is somewhat obscured. However, when the curve is surveyed in its entirety it illustrates the fact that the vitality of the organisms, as indicated by the fission rate, has maintained a higher average since the use of a promiscuous culture medium was instituted.

In order to determine more fully the effect of a very constant environment on this same race of *Paramecium* which was being maintained on a varied environment, there was isolated from each of the four lines, on February 19, 1909, at the 1,121st generation, a second culture, designated *Paramecium* I^s. This culture was submitted to as constant an environment as was practicable, according to the general method of Calkins. There were, then, two cultures of the same protoplasm running simultaneously, one being subjected to a varied or promiscuous culture medium, and the other to a comparatively constant culture medium. As a matter of precaution, and to show if there was anything intrinsically deleterious in the medium provided for Culture I^s, its constant medium was employed at various times as a temporary medium for Culture I. This, of course, simply increased the variability of the medium of Culture I. Also, near the end of the I^s series, its medium was employed not only for Culture I, but also for two cultures of ex-conjugant paramecia (*Paramecium* II^y and *Paramecium* II^z) from an entirely different source from that of the *Paramecium* of Culture I.

The results of these experiments with a constant and varied environment on the same protozoan protoplasm are shown graphically in Fig. 4. A glance at this curve shows that the vitality of the protoplasm of Culture I^s (constant environment), as measured by the fission rate, immediately fell below that of Culture I (varied environment), and that a consistent decrease in division rate was maintained until Culture I^s died out on June 6, 1909, at the 1,159th generation, after having been one hundred and seven days, or a little more than three months, on the constant medium; whereas the protoplasm of Culture I maintained about the same general average vitality throughout the period and had attained the 1,200th generation, a gain of forty-one generations in 107 days over the I^s culture. That the death of the I^s culture was not due to some sudden and accidental inimical change in the medium is proved by the fact that the same culture medium when used temporarily for the other cultures produced no deleterious effect, and also by the character of the curve of the fission rate of the I^s culture which has a consistent general downward trend except as it is affected by the rhythms. A comparison of the I^s culture curve and the curves of Calkins' *Paramecium* cultures shows a striking similarity in character. The cycles in Calkins' A culture were of six months duration and varied between 126 and 200 generations in length. My I^s culture passed through only 138 generations, but as it actually represents only the downward slope, or second half, of a cycle of Calkins' culture, my I^s cycle is really somewhat longer than those of Calkins. This point is only of interest in that it indicates in a general way the comparative similarity of the reactions of the protoplasm of paramecia from widely different sources to the same general conditions; and because it removes the possible objection that the I^s culture died out because it had been acclimated to the varied environment, and consequently it could not withstand the change to a constant medium. Of course, this is only a formal objection at best as there is every reason for supposing that the wild paramecia with which all cultures are started have been subjected for countless generations to considerably greater variations in their environment than it is possible to supply artifically.

IV. Discussion.

Up to the present time Culture I has not completed a "cycle" and all the fluctuations in vitality, as indicated by the division rate, fall under the head of "rhythms," as previously defined by me,[1]

[1] Woodruff ('05).

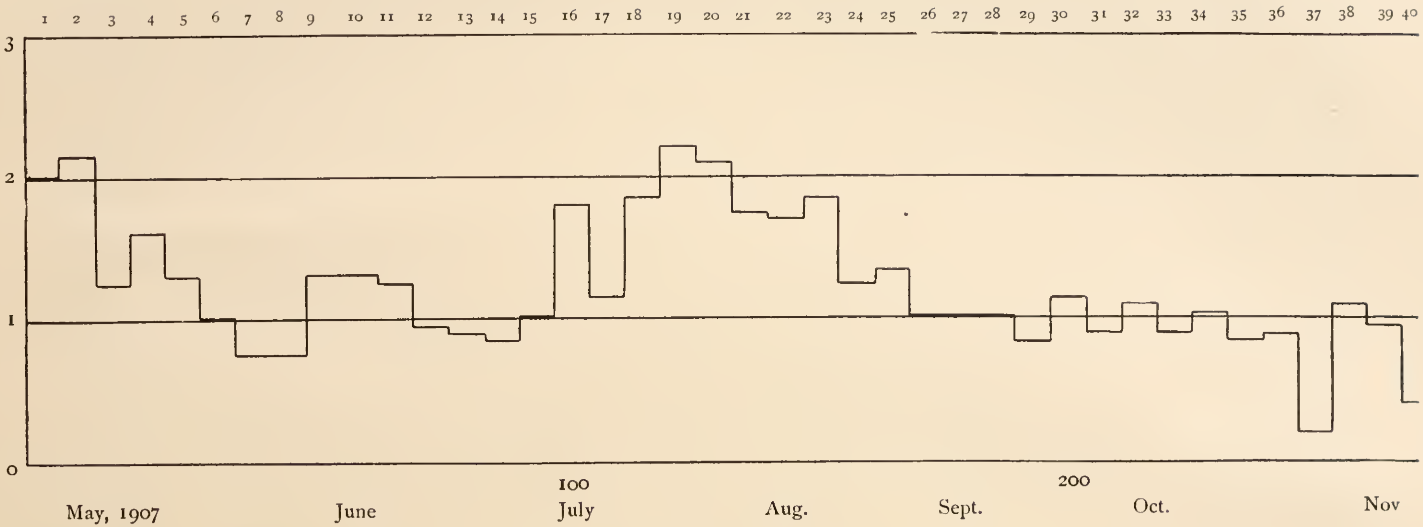

DIAGRAM III. PART I.

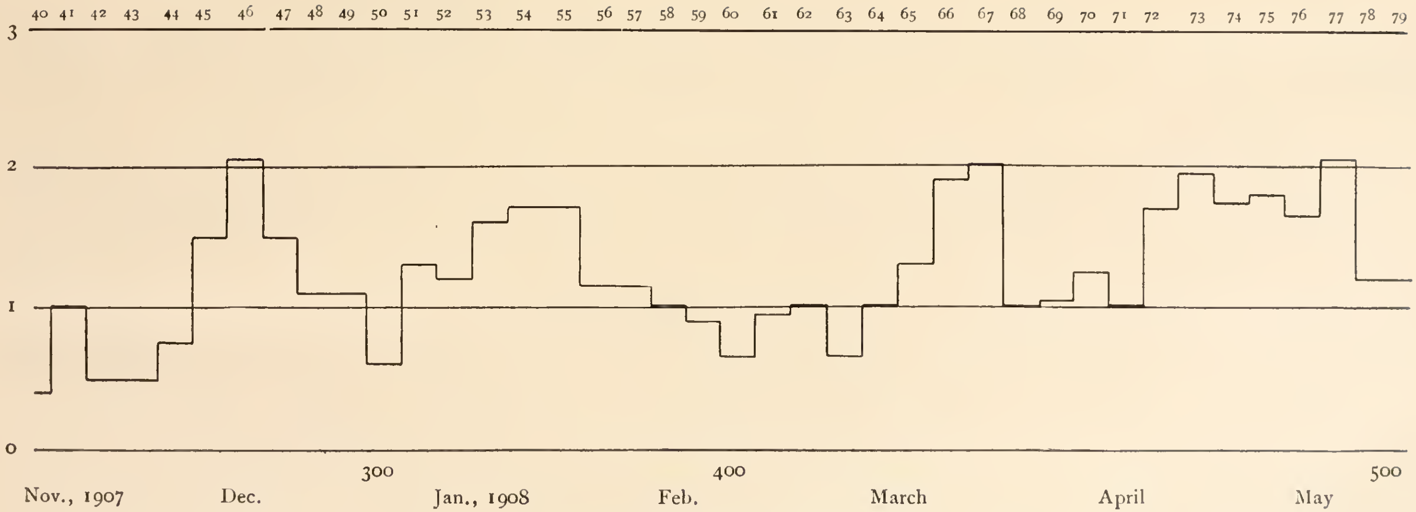

Diagram III. Part 2.

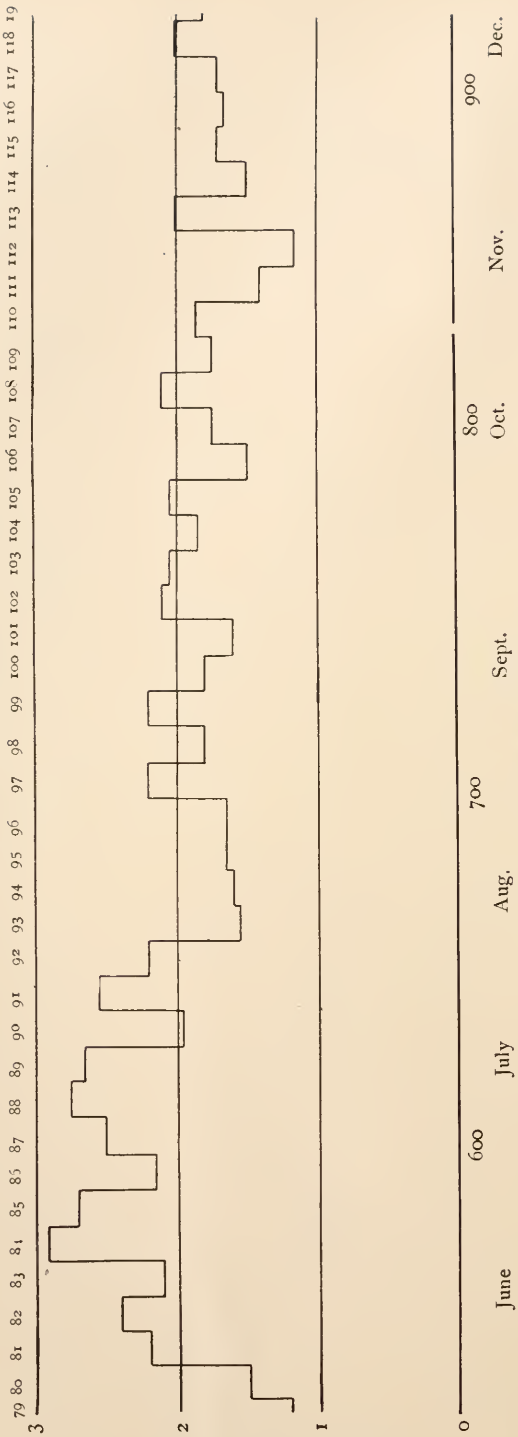

DIAGRAM III. PART 3.

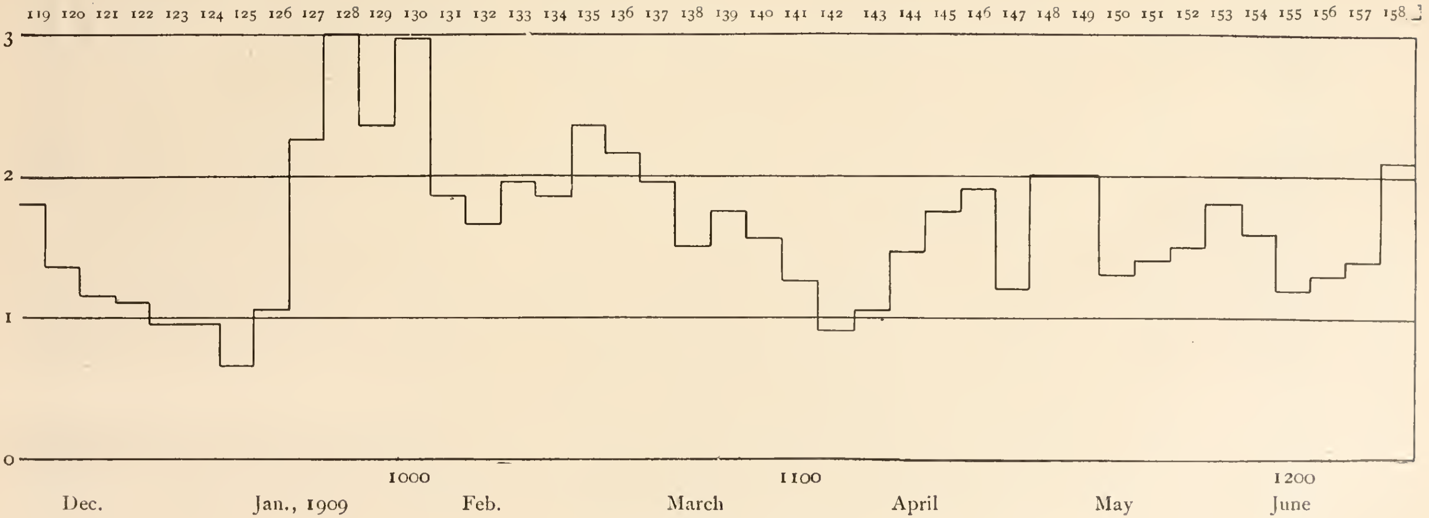

DIAGRAM III. PART 4.

FIG. 3. (Five-day periods.) Complete history of *Paramecium aurelia* (*caudatum*), Culture I, from start on May 1, 1907, to the present time, June 29, 1909, at the 1,238th generation. The rate of division is averaged for five-day periods. The ordinates represent the average daily rate of division of the four lines of the culture. Above, the numbers of the five-day periods are indicated. Below, are designated the periods in which the fifteenth day of the respective months fell. The figures, 100, 200, etc., represent generations, and are placed in the periods in which they were attained.

viz., "A rhythm is a minor periodic rise and fall of the fission rate, due to some unknown factor in cell metabolism, from which recovery is autonomous." The rhythms are more evident when a more constant environment is maintained, as I have shown in a study of the effect of a particularly stable environment on *Gastrostyla steinii*, during the months of July, August and September (cf. Fig. 5).

Gregory ('09) has plotted the curve of a *Stylonychia* culture for five-day periods from the data of Popoff ('07), which shows that the first four of the so-called "deep depression" periods emphasized by Popoff resolve themselves into "normal rhythms from which recovery is autonomous." Gregory also points out in her own 548 generation culture of *Tillina magna* that "the curve which represents the general vitality of the protoplasm shows the normal rhythmic fluctuations observed by Woodruff."

I have previously interpreted as rhythms the tri-monthly depressions in vitality, which Calkins and the earlier workers on *Paramecium* have noted, and the results obtained from my culture of *Paramecium* seem to indicate that the semi-annual cycles of Calkins are also actually rhythms, recovery from which was not autonomous under the conditions of a constant environment. The general occurrence of rhythms in the life history of infusoria is established, I believe, but to what they are due is still awaiting discovery.

Gregory has emphasized the point that "Enough consideration has not been taken of the fact that not only does each individual vary in its degree of sensitiveness at different periods in the life history, as suggested by Towle and shown by the rhythms of Woodruff, but each individual of the same species as well as of different species has its own peculiar protoplasmic reactions. Woodruff himself has failed to consider this fact in his last paper on the effects of a varied environment on *Paramecium*. . . . He cannot logically compare his results with those of Calkins for he is not dealing with the same protoplasm. . . ." In 1905 I wrote: "My cultures lead me to believe, with Simpson, that the personal equation, if I may use that term, of the individual selected to start a culture has the most influence in determining the number of generations attained. . . . Calkins' discovery of what he calls 'incipient fertilization' . . . would seem to bear out this point, and to show that the number of generations, or the period, over which a cycle extends, is not a point of great moment."

I have since found no reason to alter this opinion. But there must be *limits* beyond which the "peculiar protoplasmic reactions" of any individual do not extend, otherwise each would be a law unto itself and there would be as many laws as individuals. Certainly we may reasonably assume that there are limits of time, and generations, which a "cycle" (if it exists) of any particular species will not exceed. The earlier investigations apparently indicated that about three months or about 100 generations was the limit of the cycle of *Paramecium*. Calkins in his last paper extended the cycle to about six months, or about 200 generations. The present culture extends the "cycle" to more than twenty-six months, and more than one thousand two hundred generations. The longest culture carried by Calkins (Culture A) lived for twenty-three months, and attained 742 generations — but this comprised four complete cycles, the last one terminating fatally. It is necessary to contrast the cycle of Calkins' culture of about six months duration, and two hundred generations, with the life (cycle) of this culture, which is of twenty-six months duration at present, and 1,238 generations. That is, this culture shows a "cycle" twenty months longer in time, and, so far, of over one thousand more generations.

The *character* of the life history must also be taken into account. There is a marked difference in the character of the *Paramecium* curve after February, 1908, when the decidedly varied environment was begun (cf. Figs. 1 and 2). A similar difference in character is evident in the *Gastrostyla* culture when the more constant medium was being maintained (cf. Fig. 5, July, August and September), and the same is again strikingly shown in the present culture of *Paramecium* in the experiments which subjected the "same protoplasm" to a constant and a varied environment simultaneously (cf. Fig. 4).

The term cycle, as has been pointed out, is a relative one, but I think it is necessary to extend the conception of the cycle (as worked out on infusoria on constant media) to an unwarranted

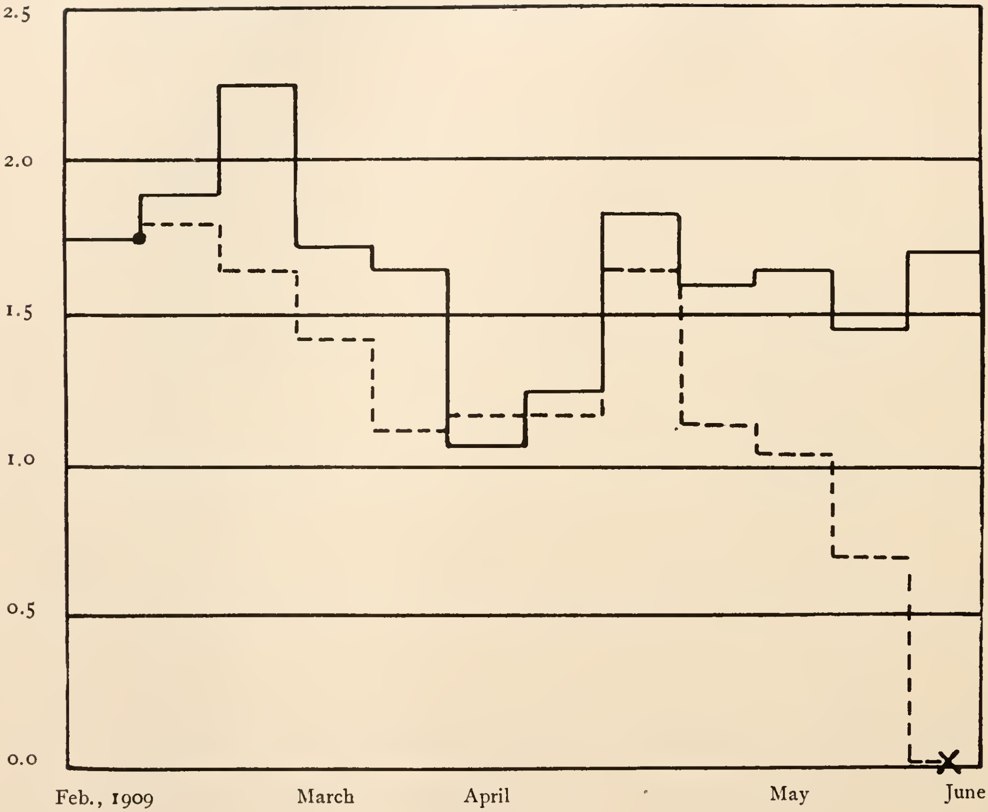

DIAGRAM IV.

FIG. 4. *Paramecium*, Culture I^s (constant environment) = broken line; Culture I (varied environment) — continuous line. ● = point of isolation of Culture I^s from Culture I. *X* = point at which Culture I^s died out. Other details as in Fig. 1.

extent if it is to be made to include the life history of the present culture. I would not suggest that the protoplasm of every wild *Paramecium* has the potential to attain over twelve hundred generations or more — undoubtedly there are strong and weak strains among infusoria as among other classes of animals. Again, it is possible that the different races of *Paramecium* which Jennings ('08) has been able to isolate may have a physiological as well as a morphological basis of distinction. It may be also that I have been particularly fortunate in my haphazard selection of culture material, so that the proper variations have been available when necessary. It is true that any particular ingredient of the infusion which might be needed would not long be available for the organisms on account of the frequent change of the character of the infusion, and Gregory has recently shown in the case of *Tillina*, and I have shown in the case of *Gastrostyla*, that daily stimulation with salts is often more efficacious than an initial stimulation. But Calkins says in regard to the second cycle of his A culture ". . . in December it was necessary to keep them on the stimulant only a day or two to get the desired result. The short treatment at this period sufficed, because they were not allowed to become weakened to the same extent as in the preceding period of depression." It is this factor which has been taken into account in this study, and it is probable that it has contributed largely to the vitality of the culture.

It must also be kept in mind that a certain amount of judgment is exercised in selecting a representative specimen for isolation. By experience one becomes quite familiar with the normal movements, shape, and general appearance of the organisms, so that it is possible to select a favorable specimen daily for the continuance of each of the lines. The precaution is nearly always taken to examine the culture again a few hours after the isolations to see how the organisms behave in the fresh culture liquid. If everything does not appear normal, a new set of individuals is isolated from the "stock" (*i. e.*, from the one, three or seven individuals left after isolation, the number depending on the rate of division during the previous twenty-four hours). Undoubtedly the process practically results in the artificial selection of the organisms which have the highest potential of division and those which are most readily acclimated to changes in their medium. Each and all of these factors may contribute to the length of the life of the culture — but after all is done the "chances" are largely against the prolonged life of the culture.

This culture suggests, then, the time-honored question whether the protoplasm of infusoria has the potential of unlimited life and reproduction, and the fundamental question as to the rôle of conjugation in the life history of these organisms. Up to the present time there has been no tendency to conjugate among the individuals of this culture, although in the "stock" cultures, consisting of the individuals remaining over after the daily isolations, there has been ample opportunity for it to take place. The daily isolations, of course, have precluded its occurrence in the four direct lines of the culture. This result agrees with those of Joukowsky on a 460 generation culture of *Pleurotricha lanceolata*, Gregory on a 548 generation culture of *Tillina magna*, and Woodruff on an 860 generation culture of *Oxytricha fallax*, on a 448 generation culture of *Pleurotricha lanceolata* and on a 288 generation culture of *Gastrostyla steinii*. Maupas secured no conjugations in his cultures of *Stylonychia mytilus* and *Oxytricha* sp., though his other series yielded plenty of syzygies. That the infusoria do conjugate is, of course, a matter of common observation; but I believe these results indicate that the phenomenon is not so frequent in the life history as is generally believed. A daily examination of twenty hay infusions, made up by several different methods, has not shown a single case of conjugation among the hypotrichous forms present either at the top or bottom of the jars. In fact, not a single syzygy has been observed in any species except *Paramecium*, and in this form conjugation has been very rare. However, a sudden transference of the paramecia from the comparatively constant culture medium of a hay infusion to a different medium has produced marked epidemics of conjugation. It is just possible that a constant medium is necessary for the so-called miscible state (Calkins) to develop, and that this becomes functional on transference to a decidedly different medium. If this is so, it may account for the absence of conjugation in my paramecia series on a varied medium, and

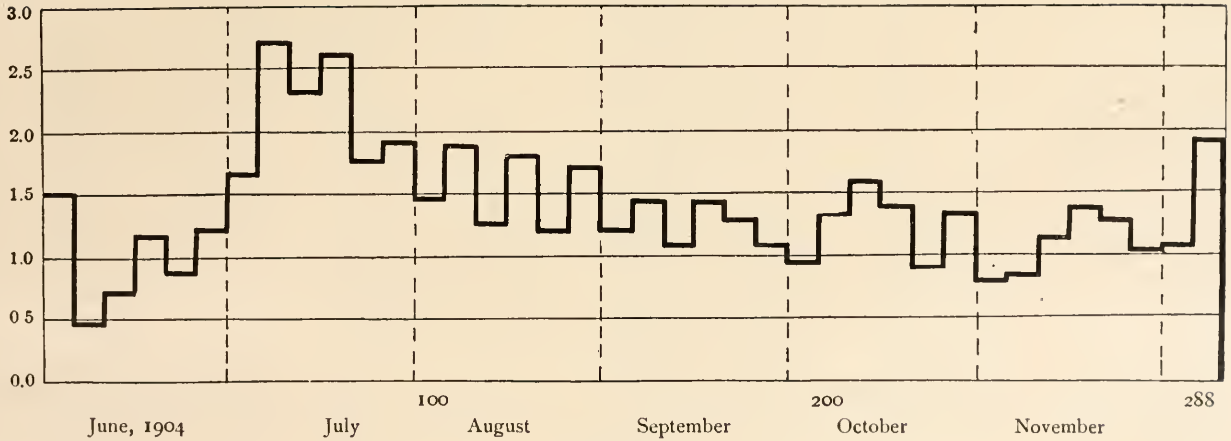

DIAGRAM V.

FIG. 5. Complete history of *Gastrostyla steinii*, Culture A, averaged for five-day periods. Method of plotting is the same as in previous diagrams.

its prevalence in Calkins' cultures on a constant medium. This idea is not supported by my hypotrichous cultures which were carried on a constant medium and still did not develop a tendency to conjugate even when the culture medium was varied in some special experiments. It is highly probable, however, that the superficial conditions which induce conjugation may vary in different species.

Periods of marked physiological depression have not appeared during the first twenty-six months of the life of the *Paramecium* I culture, but well-defined morphological changes have taken place. I shall not discuss these cytological changes at present, as I believe it is advisable to wait until the culture is terminated naturally, or by accident, so that all the data from the complete series may be discussed in its entirety. It is clear, however, that the relation of the rate of division to the so-called "normal" condition of the nuclei of *Paramecium* is not supported by this culture, as decided nuclear changes apparently do not affect the general vitality of the organisms. It may be noted further, that not a single monster due to incomplete or otherwise abnormal division has occurred in the entire 1,238 generations.

V. Conclusions.

The experimental study of the life history of infusoria has so far clearly shown that:

The protoplasm of these organisms, when subjected to a comparatively constant culture medium, passes through long cyclical changes in vitality which finally result in the death of the organism.

The protoplasm may be "rejuvenated" by suitable changes in the culture medium (stimuli) at critical points in the cycle, and thus be enabled to resume active reproduction for a longer period.

The essential fact brought out by this study is that:

The protoplasm of the individual *Paramecium* isolated over two years ago to start the culture has had the potential to divide (so far) over one thousand two hundred and thirty times at an average rate of more than three divisions every two days, and the representatives of the untold millions of its progeny which are still in captivity give every indication of being in as normal physiological and morphological condition as their ancestor. This suggests that when the protoplasm is constantly subjected to a suitable varied environment the cycle may be greatly prolonged and probably entirely eliminated — the fluctuations in vitality not transcending the rhythm.

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ON THE METHOD OF CELL DIVISION IN TÆNIA.[1]

A. RICHARDS.

Within the last few years the question of the significance of amitosis has pushed its way forward with renewed activity. From the classic views of Ziegler and vom Rath, Flemming and others that amitosis may be expected in unicellular organisms, in degenerating and senescent cells, and in highly specialized and pathological tissues opinion in some quarters has departed widely.

On the one hand the tendency has been to narrow this view. In many of the Protozoa mitosis has been found quite general, at least in some stage of the life cycle, while in certain Rhizopods, as *Arcella* and *Euglypha*, where direct division was formerly thought to be the means of reproduction, it is now known that mitosis is the common method. In the case of highly specialized cells numerous examples are reported in which careful study has shown mitosis as the chief means of division. An example of this tendency is seen in Strasburger's work on the tapetum cells. He found that while the period of mitotic division was very short it was sufficient to account for all the observations that had hitherto been explained on the basis of amitosis. Again the application of improved cytological methods of fixing and staining have thrown into disrepute, to a large extent, the old view of the occurrence of direct division in pathological tissues; indeed the phenomenon of reduction has been described in cancer cells. On these lines of research, then, the tendency has been to limit our notions of the rôle of amitosis in nuclear and cell division.

On the other hand more recently a new line of reasoning has been developed, perhaps more rapidly than the facts warrant. This new line, of which Child is the chief, although not the first exponent, is to the effect that direct nuclear division occurs in rapidly dividing cells and in cases in which an orthodromic or acyclic process is involved. In this connection the statements of several writers that mitosis may follow amitosis are to be noted in which they cite cases of direct division in the maturation and prematuration stages of various forms. To meet these claims we shall doubtless have to revise somewhat our ideas of the meaning of amitosis, but at present the progress toward such a revision seems to have overstepped the bounds of conservatism.

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[1] Laboratory of Zoölogy, University of Wisconsin, June 4, 1909.

Among the older workers on this line are Meves, Preusse, and Pfeffer. Meves found amitosis to occur in the early stages of spermatozoön formation in *Salamandra* in the autumn followed by mitosis in the spring, but some of these cells have since been shown to take no part in the formation of spermatozoa. Preusse's work has been much quoted in this connection. He found amitosis in the ovaries of Hemiptera. However, a reinvestigation of this case by Gross in 1901 served to bring it under the theory of Ziegler and vom Rath. Gross showed that this method of cell division did occur but much less widely than described by Preusse. Its occurrence is restricted to two kinds of cells, degenerating and secretory; this, of course, proved that he was dealing with a special case under the old theory. Pfeffer's work on *Spirogyra* has been discredited by Nathansohn; in fact, opinion among botanists is decidedly adverse to the view that amitosis may be followed by mitosis in a single nucleus. This opinion is expressed by Strasburger in his recent summary of the individuality question.

Working on the spermatogenesis of the sparrow in 1900, Loisel saw nuclei which began division by amitosis and later continued by indirect division. He says that the amitosis was not a sign of degeneration; but again, he shows that the greater part of certain spermatocytes and spermatids degenerates. To reach a safe conclusion in this case one must needs know the relation between amitosis in the sex cells and degeneration in the sex products. Degeneration on the part of spermatozoa in the Hemiptera has been traced by Morgan and by Miss Stevens to the absence of a single chromosome. If cells lacking a single chromosome degenerate, certainly one would expect degeneration in cases where part of the sex cells had previously divided by as indifferent a method as amitosis seems to be.

Especial importance has been attached to cases of amitosis in regulatory growth. G. T. Hargitt was the first to suggest this for hydroids. Child's work on *Tubularia* and *Corymorpha* supports this suggestion. In the growth and regulation of *Planaria*, Bardeen and Child have reported amitosis. However, the figures of Bardeen are far from conclusive, and it is very questionable whether they justify the opinion that amitosis is the method of division here. Child has also worked on various other forms among both vertebrates and invertebrates. In several of them his evidence is lacking in some respects. Reference to his work on the cestodes will be made later.

A few workers have described amitosis in the cleavage of the egg and in the early embryonic development of several forms. Hargitt failed to find mitosis of the egg up to the sixteen-cell stage, working on *Clava leptostyla*. Similar results obtain for *Eudendrium* and *Pennaria*. Beckwith, however, has recently shown that his results were due "simply to the fact that the eggs were not obtained at the right time of day. In eggs collected at the proper time (4 to 6 A. M.) there is no difficulty in proving the typical stages of maturation and fertilization." "Maturation and the early cleavages take place by mitosis and not by amitosis." Hickson and Hill have also studied cœlenterate eggs. Hill in his account of *Alcyonium* oögenesis shows that no polar bodies are extruded, no chromosomes are present, the female pronucleus divides irregularly by amitosis and then disappears, and that probably the first cleavage nucleus is formed from the male pronucleus. The evidence is not complete and the case should certainly be reinvestigated. H. L. Osborne described cases of amitosis in the food-ova of *Fasciolaria*. His results have been corrected and enlarged upon by Glaser. The work of Glaser seems to deserve the most careful consideration in regard to this problem; its bearing on the investigation herewith undertaken is only general, however. Further work on embryos has been done by Child, previously mentioned, and by Patterson on the pigeon's egg. The observations of the latter, while much more extensive than those of Child on the chick embryo, are in agreement with them. Stoeckel thought binucleate ova in man are the result of amitotic divisions. Fick's opinion on the subject of amitosis as expressed in his survey of chromosome hypotheses is based upon the work of Child and Hill and upon his own *a priori* conclusions. He offers nothing new on the problem.

Direct division has been described in other cases of theoretical importance but those mentioned above are perhaps the most significant.

METHODS.

My investigation on the problem of amitosis was suggested by Dr. S. J. Holmes, to whom I owe much for direction during the progress of the work. I have received numerous suggestions from various other workers in the University of Wisconsin, all of which are gratefully acknowledged. My thanks are also due Drs. Grove and Meek, of the Pharmacology and Physiology Departments, for their assistance in collecting material.

Specimens of tape-worms were secured chiefly from dogs. A considerable number of cats was examined, but only one furnished material. The specimens were nearly all fixed in Flemming's fluid, which proved quite satisfactory. Those taken from the cat were fixed in Zenker's fluid to be used with Mallory's connective-tissue stain.

A variety of staining methods was used. Flemming's tricolor stain did not give sufficient sharpness of detail to be of much value. Iron hæmatoxylin is in general satisfactory, but it is to be noted that the nuclei do not differentiate as readily as in many other tissues. The fact that they do not decolorize readily and often do not show their contents clearly must be borne in mind when considering the significance of indentations of the nuclear membrane. Delafield's hæmatoxylin decolorized in acid alcohol gave excellent results. My greatest success, however, in staining this material has been by the use of Kernschwarz with Lichtgrün as a counterstain. Lichtgrün is by far the best stain for cytoplasmic structures that I have tried, its only drawback being the ease with which it fades out.

Two genera and three species of tape-worm have been used: *Tænia marginata* (Batsch), *Tænia serrata* (Goeze), and *Dypilidium caninum* (Leuckart). The last of these is the most favorable for cytological work, but I have only a few specimens of that genus. Even here the nuclei are quite small and not entirely satisfactory owing to technical difficulties. I must protest against the balancing of results obtained from such unfavorable material as that which the cestodes offer against such favorable objects for cytological study as, for example, the Orthoptera. In the cestodes which I have studied, the cells, except the oöcytes, are much smaller than the insect cells, do not stain as readily, and are often obscured by great masses of intercellular material.

Aim.

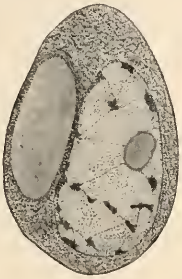
My aim in this investigation was to obtain definite evidence as to the occurrence of amitosis in cestode tissues. Observations were begun with the hope of bringing into line, in a small measure at least, the account of cell division in this group with the results generally obtained by workers on other forms. Lack of time has prevented the investigation of many of the secondary questions that have arisen. Thus no attempt has been made to give details of chromosome behavior or structure, and the observations have been limited to the method of cell division in the process of oögenesis and in the growth of somatic cells. The discussion, however, includes occasional reference to related questions.

Observations.

Oögenesis. — The female sex cells in the cestodes in question are by far the largest cells in the body. They are in general round with a relatively large nucleus. The cytoplasm is fibro-recticular and to a certain extent granular. Occasionally large dark granules appear, as in Figs. 15, 16 and 17; their nature has not been definitely made out, but they may be yolk nuclei or, perhaps, nothing more than aggregations of smaller cytoplasmic granules. Frequently they serve to obscure the process of mitosis.

No cell organs are located in the cytoplasm of the resting mother cells; but, at some time during the development of the ovarian egg, a mass, probably of yolk, appears there. I have not observed any regularity in the formation of this mass, for some of the early oögonia have it, while it is not present in some oöcytes. The masses, of course, vary in size. Those which are newly formed have a close resemblance with certain stains to a "nebenkern," and, in fact, have been so called. They were first described by Sommer in 1874, and named by him "Nebendotter." This expression, which has the claim of priority, seems unobjectionable except on the score of bringing a foreign word into English; no suitable translation has been suggested, however. On the other hand, the body is not a true "nebenkern," and to call it by that term is a misuse of the word.

Fig. 1 is an early oögonium from *T. serrata.* Here the "Nebendotter" appears as an egg shaped body of even consistency stained darker than the nucleus although lighter than the surrounding cytoplasm. Its reactions to various stains deserve metion. With iron hæmatoxylin it stains readily, appearing as a dark homogeneous mass even after a great deal of extraction of the stain. The nucleus and cytoplasm may be entirely decolorized and the "Nebendotter" still show as a dark body, a fact which led to confusion during the early part of my study, as the nucleus was overlooked and the "nebendotter" taken for it. The true state of affairs was not revealed until I had used another method of staining when the appearances which with iron hæmatoxylin had misled me were explained and the structure of the cells became clear. The new reagents were Kernschwarz counterstained with Litchtgrün. Kernschwarz is a weak stain affecting only the nucleus. In my preparations I have seen no trace of it in the cytoplasm or in the "Nebendotter." Lichtgrün stains both nuclear plasm and cytoplasmic structures. The result, then, with this method of staining is as follows: chromatin and nuclear



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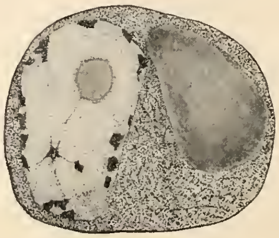

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FIG. 1. Oögonium, stained with iron hæmatoxylin, showing "Nebendotter," and nucleus with chromatin reticulum.

FIG. 2. Oögonium showing same structures as Fig. 1, but from a much later generation.

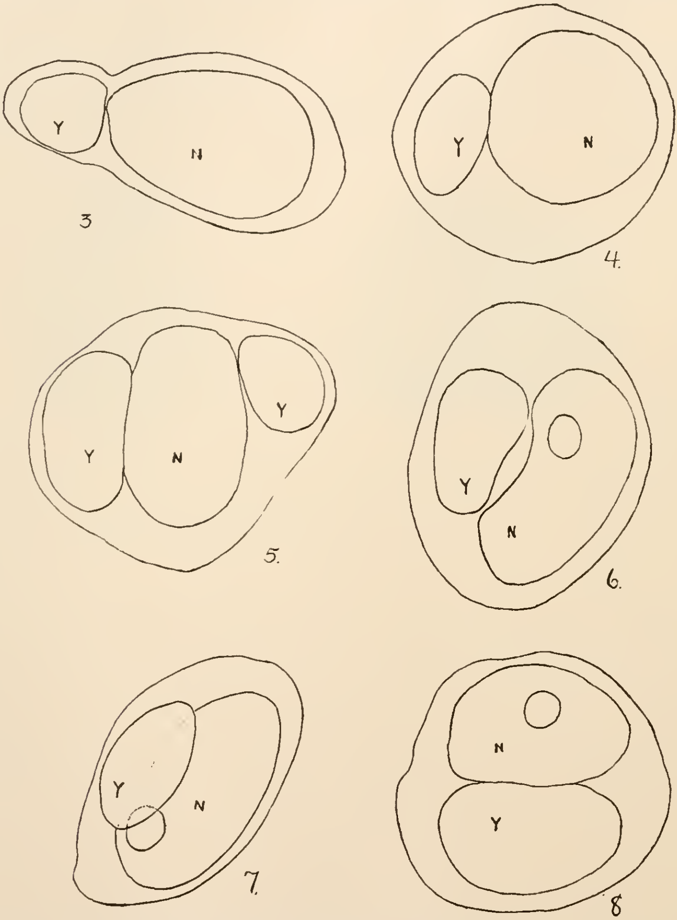
reticulum, blackish; nuclear plasm, very light green; general cytoplasm, dark green, fibro-reticulated; "Nebendotter," homogenous "cheesy" green. This last appearance is very difficult to describe but is recognized very easily.

The "Nebendotter" as such has not been described by Child. One is compelled to suspect that the same appearances which confused me may have misled him. Occasionally a constriction is seen in a "Nebendotter" or there may be two or more distinct yolk masses in a cell. More often, especially in the case of hæmatoxylin slides not well decolorized, the "Nebendotter" does not look unlike a dividing or divided half of the nucleus. Child figures cases in which one half of the nucleus stains darker than the other half. Is this darker half perhaps a "Nebendotter"?

To illustrate the above facts a series of outline drawings is given. They were made with the aid of a Zeiss No. 5 ocular and a Leitz one twelfth objective (oil immersion). In each case *n* represents the nucleus and *y* the "Nebendotter." In studying these figures one can easily see how refractive properties may have obscured the boundaries between the various parts. All figures in this series are of resting cells such as are shown in Figs. 1 and 2. Figs. 3 and 4 resemble cases of unequal constriction of the nucleus, Fig. 8, of equal constriction, and Fig. 5 a nucleus divided into three parts by amitosis. Fig. 6 suggests that division began at the center and progressed outward. Compare these figures with those from Child's paper on oögenesis (6): Fig. 1 with his Fig. 29; Fig. 2 with his Fig. 9, *b*; Fig. 3 with his Fig. 8, *b*; Fig. 6 with his Fig. 11, *A*, *a*; Fig. 7 with his Fig. 13, *A*; and Fig. 8 with his Fig. 10, *A*. The similarity is very suggestive.

Resting nuclei are seen in Figs. 1, 2, and 9. Fig. 9 is lacking in a "Nebendotter." The nuclear plasm in the resting condition seems homogeneous throughout and takes a very light stain. The nuclear membrane is a very delicate structure, showing as a thin line in some cases, while in others its location is marked only by the inner edge of the cytoplasmic reticulum. A nucleolus is usually present in the early stages of cell formation; it takes a very light stain in some cases resembling the "Nebendotter." I have never seen a divided or dividing nucleolus.

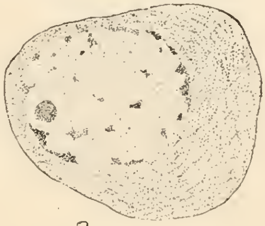
With regard to their chromatin content the nuclei of *Tænia* differ from those of *Monieza*. Child states that "the only deeply staining portions of the nucleus up to this time (end of oögonial division period) have been the nucleolus and frequently a few other granules." In another place he says that the nuclei do not contain any definite reticulum. That his statements do not hold for *T. serrata* may be seen from my Figs. 1, 2 and 9. The chromatin content is small in amount, but it can be seen in definite masses which are scattered over the periphery of the nucleus and which are connected by definite strands of linin. The reticu-



FIGS. 3–8. Outline drawings from oögonia showing various relations assumed by the nebendotter and nucleus. *N*, nucleus ; *Y*, "Nebendotter."

lar character of the chromatin and linin is clearly shown with Kernschwarz and Lichtgrün. This description answers not only for late oögonia but also for the early ones and for the oöcytes. Fig. 9 is an early oögonium.

Fig. 10 illustrates the early condition of the spirem stage. Oögonia in this stage and slightly later are very numerous in certain lots of material. In other lots I find many resting stages. Anaphases and telophases, too, are not difficult to find but metaphases are conspicuously absent. This probably means that the metaphases are of short duration. It also indicates a fact which I believe to be very pertinent to the question of the frequency of mitoses; namely, a periodicity[1] with regard to the divisions. It is well known that physiological factors may govern the time of mitotic divisions. A case in point is that recently described by Beckwith, previously mentioned; likewise, in certain insects and in many plants mitosis occurs at night only. The fact that many nuclei from one lot are in the same stage of division indicates, I believe, the effect of some physiological factor. What that factor is, I can only conjecture. Perhaps mitotic periods may occur only after a more or less prolonged fast on the part of the host, for then the energies of the parasite are not directed towards the assimilation of food.



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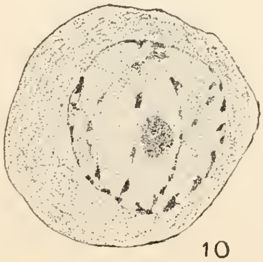

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FIG. 9. Resting oögonium with a definite chromatin reticulum; no "Nebendotter" present.

FIG. 10. Oögonium; very early prophase, spireme formation beginning.

FIGS. 9–17. From cells stained with Kernschwarz and Lichtgrün.

During the maturation period the regular course is followed.

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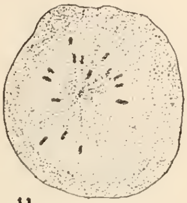
[1] This expression is not intended to imply that a definite amount of time intervenes between successive periods; they may recur at irregular intervals depending on some physiological factor.

Figs. 13 to 17 illustrate the process, but no attempt is made to give even a meagre outline of the behavior of the chromosomes in this period. However, attention is called to the appearance of the spindle. While spindle formation is quite regular, the achromatic fibres do not stain well and frequently the entire structure is overlooked. This fact taken with the smallness of the chromosomes and the prominence of the large cytoplasmic granules may well serve to veil the process of mitosis. In many cases one does not, at first, distinguish between the chromosomes and the cytoplasmic granules, so nearly alike may they appear. While Fig. 15 shows clearly its mitotic character, the cell from which it was drawn was overlooked for a long time.



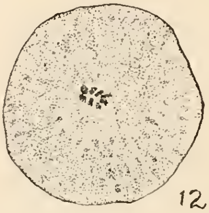


FIG. 11. Oögonium; polar view of an anaphase. This figure and the next are from early generations of oögonia.

FIG. 12. Oögonium; polar view of a telophase.

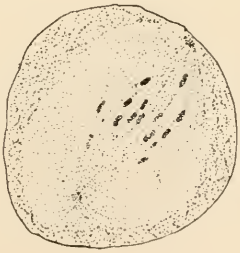
Fig. 17 is a case which suggests an "endogenous" division. According to Child, an endogenous division is that of a nucleus into two nuclei within the old nuclear membrane. Upon a superficial inspection the cell in question seemed to be in process of such division as shown by the outline in Fig. 18. Careful study, however, revealed the mitotic nature of the division. It is a late telophase with the chromosomes disintegrating; remnants of asters may be seen, as can a Zwischenkörper.

The appearance of this cell suggests the question of the relation between nuclear and cell division. Botanists have recognized the distinction between the two processes much more generally than have zoölogists. The latter have been accustomed to regard nuclear division as a sign of immediate cell division. Very often this is not the true state of affairs, for nuclear division may never be followed by cell division (Marshall), or a considerable period of time may elapse before a cell plate is formed. Fig. 17 shows no sign of cell division although mitosis is almost complete. This is not an infrequent occurrence; actual nuclear division and certainly cell division may lag well behind spindle and chromosome division. Herein lies a fruitful cause for misinterpretation. A nucleus in which chromosome division has been completed would give every appearance of direct division upon constriction and subsequent division. Two nuclei in a single cell which had not begun to form a cell plate would also be misleading.

No true case of amitosis has been observed in the egg cell formation of the *Tænias* upon which I have worked.

Somatic Tissues.—The somatic tissues of tape-worms inside the cuticle include muscle fibres, excretory and genital organs, and a primitive nervous system. Surrounding all of these organs and filling all interstices is a large mass of parenchyma. A detailed study of all of these structures has been made by Child for *Monieza*. He reports that many cases of amitotic division occur and that illustrations of this fact might be multipled indefinitely.

I have made no investigation of the method of cell division in the excretory or nervous systems of *Tænia*. There is no reason to believe, however, that the method of division in those organs differs from that of the genitalia or of the muscle fibers. We have in all of these systems cells that are specialized to a high degree. Even if amitosis be found here where rapid growth may be taking place that fact loses significance when the degree of specialization is considered. The various cell generations of a differentiating tissue differ from earlier generations only in a gain



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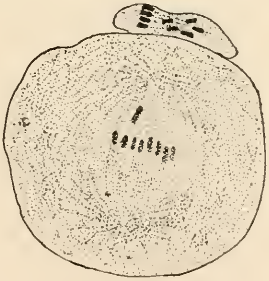

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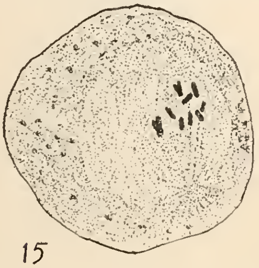
Fig. 13. First oöcyte; late metaphase.

Fig. 14. Second oöcyte; equatorial plate stage, first polar body dividing.

in specialization and a loss in reproductive potentiality. Rapidity of division in these cases is a negligible factor. We are simply dealing with a special case under the old theory, if amitosis be found to obtain here. Amitosis, then, may well be expected in these systems.

The facts observed in the genitalia of *Tænia* do not bear out fully that expectation. The structure of the genital ducts and organs can be made out clearly and nuclei, cytoplasm, and intercellular substance seen. Yet cases of amitosis have not been demonstrated. On the other hand, a mitotically dividing nucleus is found only rarely. This may mean that mitosis is of very short duration, or more probably, that it is of short duration and occurs in waves; or, again, it may indicate that the nuclei divide amitotically. But whichever interpetation we may accept we do no violence to the theory of Ziegler and vom Rath.



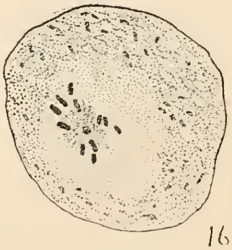


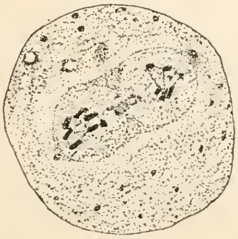
FIG. 15. Second oöcyte; early anaphase, spindle stained very lightly. Figs. 15–17 show the large cytoplasmic granules.

FIG. 16. Second oöcyte; telophase.

The muscle-cells also furnish only negative evidence of amitosis. They are large, spindle-shaped cells from which contractile fibers extend. The cytoplasm is densely reticulated, rarely exhibiting the vacuolated structure described by Child (8). The quantitative relations of cells and fibers at different periods of development are of interest. Relatively more muscle cells are present in a young proglottid than in an old one, but the muscle fibers are much more developed in the later stages. The significance of this relation, which agrees closely with a similar parenchymal relation, will be discussed later. While satisfactory evidence as to the usual method of cell division has not been obtained, the observations on the material at hand favor mitosis as typical.

The meaning of amitosis in the parenchyma is of quite different import from that in specialized tissues. Parenchyma is a tissue from which others are derived; it is neither highly specialized, degenerating or pathological. The occurrence of amitosis here, therefore, would not be in line with the old theory.

Concerning the nature of parenchyma there has been much controversy, but it is beyond the scope of this paper to enter into a discussion of the question or of the literature regarding it. In connection with an account of the method of cell division, however, a few observations concerning it cannot be avoided. The older view held by Leuckart and his followers was that the parenchyma develops from rounded and polyhedral cells, the latter sending out processes which interlace about the former. Moniez and his followers offered an opposed view. Opinion has by no means become settled even now. Child speaks "of the parenchyma cells if the syncytium which composes the parenchyma can properly be said to be composed of cells."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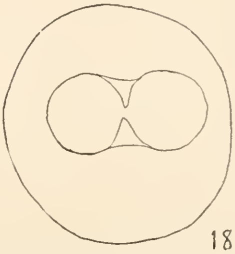
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FIG. 17. Second oöcyte in which mitosis is nearly complete, yet no sign of cell division. The daughter nuclei are being constricted apart and the chromatin masses are disintegrating. Zwischenkörper present.

FIG. 18. Outline drawing of Fig. 17. It gives the appearance of an "endogenous" division by amitosis. Fig. 17 shows that appearance to be entirely superficial.

Parenchyma consists of calcareous bodies and irregularly shaped cells lying in a mass of material which seems to be intercellular. Of these, the first do not enter into the question under consideration, and the intercellular matter only figures as a ground substance in which the cells are imbedded. The cells vary in shape from elliptical and spindle-shaped to an irregular form with many protoplasmic processes. In size there is also much variation, due, largely, to the variation in the amount of the cytoplasm, for the nuclei do not exhibit any such striking quantitative differences as does the cytoplasm. The cells do not seem to form a syncytium of which the ground substance is a part, as some writers have stated. The evidence seems to me to indicate that the ground substance bears a relation to the parenchyma cells similar to that borne by the intercellular matter of connective tissue, for example, to the connective-tissue corpuscle. That the parenchyma cells have definite boundaries is brought out clearly in the Lichtgrün preparations. I have seen no evidence for thinking the ground substance continuous with the cytoplasm. The nuclei of the cells show uniformity of structure as well as of size; they have a chromatin reticulum and usually a nucleolus. The parenchymal cell is thus seen to be a definite structure with a typical nucleus and a varying amount of cytoplasm.

Fig. 19 is a resting parenchyma cell of characteristic appearance. The cytoplasm is drawn out into strands upon the number and size of which depends the width of the cytoplasmic band about the nucleus. Parenchyma shows regional differentiation in the relative amount of ground substance and cells and in the modification of the cytoplasmic parts of the cells only. In the younger cells the cytoplasmic strands are less numerous and frequently extend only a short distance. In older regions more cytoplasm is drawn out into the strands, leaving but a thin layer about the nucleus.

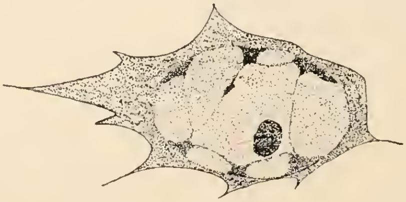

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Fig. 19. A typical parenchyma cell in the resting condition. This cell is from a proglottid in which the sex organs are only partially developed.

Professor Child has assumed through all his work that the absence of mitotic figures in tissues known to be growing rapidly is evidence of the occurrence of division by amitosis. This assumption is, of course, based on good *a priori* reasoning, but I do not believe that it is borne out entirely by the facts. In *Tænia* the parenchymal cells are relatively less in number in the old tissue than in the younger, a relation which is only partially explained by the fact that other kinds of cells develop from the parenchyma. On the other hand, the ground substance in the young proglottid is more spongy and less in amount than in the older portions of the animal. These observations show that the growth of the parenchyma is not due alone to cell multiplication but also to the formation of new intercellular matter and to the greater development of the cytoplasmic strands. The growth of the cestode soma is due chiefly to the growth of the parenchyma and of the muscle cells and fibers. Other tissues, except the sex products, are practically negligible in accounting for increase in size. With regard to the growth of the muscle fibers a similar condition obtains in them as in the parenchyma, as is shown above. Thus we find growth depending not so much on the increase in the number of cells as in the increase in amount of products of cellular activity, that is, in fibers and intercellular material. Therefore, the rapidity of growth in the cestode body does not necessarily postulate a large number of dividing cells.

As to the method of cell division little can be said. No traces of amitosis appear. Cases of several nuclear divisions in a common cytoplasmic mass such as are figured by Child (11) for *Monieza* are not to be seen in *Tænia*, nor is any explanation of them afforded by the latter genus. I have also observed no cases of mitosis. The evidence at hand, I believe, does not warrant any conclusion as to the method of cell division in the parenchyma of this form.

SUMMARY.

1. Amitosis has not been found to occur in the oögenesis of the cestodes studied.
2. All observations on the process of oögenesis point to mitosis as the usual method of cell division.
3. The presence of a "Nebendotter" which has peculiar staining properties gives a misleading appearance of amitosis.
4. Maturation is of the typical form.
5. Indirect evidence strongly suggests that physiological factors influence the frequency of mitotic divisions.

6. Nuclear divisions are not always followed immediately by cell division.

7. Only negative evidence as to the method of division in somatic structures has been found; there is no satisfactory evidence of amitosis and mitosis is not abundant.

8. Rapidity of growth in the somatic tissues of the cestodes body does not necessarily postulate many division figures.

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Vol. XVII. *October, 1909.* *No. 5.*

BIOLOGICAL BULLETIN

SOME EGG-LAYING HABITS OF AMPHITRITE ORNATA VERRILL.

JOHN W. SCOTT.

Several years ago while engaged in working out the development of the unfertilized eggs of *Amphitrite* I had occasion to collect large numbers of these worms. Mead, who worked on the cell-lineage of this annelid, had stated that nothing was known of their breeding season but that ripe sexual products could be had at irregular intervals during June, July and August. Collecting therefore during 1902 and 1903 was entirely at random. Incidentally the opportunity came to observe something about their egg-laying habits. During 1907 and 1908 I have used eggs of the same species for the study of other problems and have had a chance to further verify and extend the observations previously made. I wish here to express my appreciation to the directors of the Marine Biological Laboratory for their kindness and encouragement in helping me to prosecute this work.

The observations mentioned in this paper pertain to two questions in particular. I have always experienced considerable difficulty in obtaining, when wanted, mature sexual products of *Amphitrite*. The first question therefore deals with the time of egg-laying, in the hope that future investigators on this species may be saved some trouble and disappointment. The second question is concerned with the manner of depositing eggs. The eggs and sperm float free in the body cavity and these products are usually in various stages of development. This is true even at the time when worms deposit the mature products in a manner apparently normal. How is it possible to retain the cœlomic corpuscles and the unripe eggs and deposit the ripe sexual products? In a number of instances I have observed the act of depositing eggs or sperm in the laboratory and I studied especially the manner of depositing the eggs.

My results in general are as follows. First, the egg-laying reflex is closely associated with the time of spring tide, the height of the season occurring at the time of new or full moon, or within two days after these dates. The best results were obtained in July, *i. e.*, a larger percentage of mature worms may be collected during this month than during any other. In early summer the period of sexual activity tends to occur a day or so later than the time mentioned, while in late summer the period tends to be earlier by the same amount. In regard to the second question I may say that the ripe and unripe products are not kept separate in laying with absolute exactness. Though among the first few hundred eggs deposited it is hard to find even one that is immature, toward the close of any given period of oviposition the immature eggs form a considerable percentage of the total number. But how does the worm keep these eggs apart in the first part of the period? A full consideration of this question is given later.

In order to understand the discussion of the two questions concerned it will first be necessary to say something of the habitat of this form and the environment under which it lives. These worms live in U-shaped, rather tough, mud tubes that break easily in digging. At one of the openings of the tube, sometimes at both, there is a volcano-shaped mound of sand or earth. The two openings are ordinarily from ten to eighteen inches apart and the depth of the lowest part of the tube is about three fourths the distance between the openings. The worms were collected in six different localities in the vicinity of Woods Hole. In these localities at the time of spring tide extreme high water and extreme low water differ by two to three feet. The tubes are found most abundantly on sandy flats which may vary from fine sand to a rocky character; they are also occasionally formed on sandy mud flats where the tide produces little current. The vertical distribution of the tubes is also comparatively limited. At the time of extreme low water tubes are rarely found beyond a depth of twelve or fifteen inches, and very few are found more than twelve inches above this line.

Probably two thirds of the tubes are within six inches, in a vertical direction, of this low water line at spring tide. The worms are more abundant on flats which are somewhat protected from strong currents. When the tide is running and the water comparatively shallow, it is quite common to see the worms at one of the openings, apparently feeding, the mouth just below the pit of the "volcano," and the numerous tentacles extending out for several inches in radial directions. It was found impossible to observe the deposit of sexual products under natural conditions, and this careful description of their habitat is given in order that we may better interpret the results obtained in the laboratory.

I. Time of Egg-Laying.

Verrill in 1871–2 described the occurrence of an annelid in Vineyard Sound to which he gave the name *Amphitrite ornata* Verrill. Several years later he gave the credit for the original description to Joseph Leidy who in 1855 had described what appears to be the same species under the name of *Terebella ornata* Leidy. No mention is made of egg-laying habits in either of these papers. To Mead belongs the credit of recording the first observations of this kind. He writes : "The limits of the breeding season are unknown. Although about eight hundred worms were collected in lots of twenty or thirty between the first of June and the last of August, only seldom were ripe eggs and ripe spermatozoa obtained. It is useless to cut the worms open, for if the sexual products are mature, they will be discharged, usually at about six o'clock in the evening, more often on the day of capture, sometimes the next day." I found little difficulty in verifying most of these results.

Amphitrite must necessarily be collected in the day time and when the tides are low. They were collected in quantities, from twelve to seventy-five specimens in each lot, and it is estimated that about two thousand adult worms were examined in the course of the four seasons during which their habits were under consideration. When dug they were washed free from the mud tube and placed in a bucket of sea-water to be carried to the laboratory. At times the males and females were placed in separate buckets, but this practice was not often followed, for placing the sexes together did not cause them to discharge sexual products. In the laboratory all worms were carefully washed and isolated in separate dishes of sea-water. Eggs rarely fertilize if removed by cutting open the body wall ; if any considerable number are mature they will later be discharged in an apparently normal manner. The quotation from Mead, concerning the scarcity of ripe eggs and ripe spermatozoa, gives an accurate idea of my own results in that particular. But the time of discharge appears to be not so definite as one might think if guided solely by his description. Table I.

TABLE I.

Time.	A. M.			P. M.										Total.
	9	10	11	12	1	2	3	4	5	6	7	8	9	
Females........			1	1		2	2	3		3	3	1		16
Males..........							1	3		2	2	1		9
Total			1	1		2	3	6		5	5	2		25

Showing time of day when *Amphitrite* begin to deposit sexual products. In the earlier work no record was kept of the males, hence the small number given in this table.

gives little support to his statement in regard to the time of day at which the worms deposit their eggs. As will be observed twelve of these worms, nearly one half of the entire number, deposited their sexual products between 6:00 and 8:30 P. M. An almost equal number shed their products between 2:00 and 5:00 o'clock, and two specimens were ovipositing between 11:00 A. M. and 1:00 P. M. Injury will sometimes cause *Amphitrite* to throw off their eggs, and of course it is possible that these two worms were injured in some way. However another fact must not be overlooked. It was noticed that nearly all worms deposited products from three to five hours after the low tide at which they were collected. This fact is undoubtedly important and probably explains some of the discrepancies found in the table. In a few instances collections were made between 5:00 and 8:00 A. M. ; more frequently they were brought to the laboratory about noon ; but, owing to local conditions, by far the largest number of worms was collected when the low tide occurred in the afternoon. I believe this tendency to deposit eggs in the afternoon or early in the evening may be accounted for in the following way. When low tide occurs near mid-day or early afternoon, the sand flats are more exposed and reach a higher temperature than under ordinary circumstances. Metabolic changes are undoubtedly more active at these times and for a few hours immediately following. As a consequence, if the worms behave in their tubes as they do in the first few hours in the laboratory, the eggs are laid on a rising tide about the time of slack water. This time would be favorable for fertilization on account of the absence of strong currents. Some five hours later, the young blastula is ready to swim, as shown in a previous paper ('06).

TABLE II.

	Days Before.							Spring Tide.	Days After.							Total.
	7	6	5	4	3	2	1		1	2	3	4	5	6	7	
Females						2	1	6	2	2	1		1		1	16
Males						1	1	4	2	1	1					10
Females (injured)......								2	1				1	1		5
Males (injured)						1		2	1		1		1		1	7
Total..............	0	0	0	0	0	4	2	14	6	3	3	0	3	1	2	38
Number of times collected......	3	4	1	5	4	5	3	7	4	5	6	6	3	3	6	65

Shows time at which eggs and sperm were deposited in reference to spring tide. Also the number of collections made in reference to the same period.

In all sixty-five experiments are here recorded, scattered pretty evenly throughout the summer from June 24 to August 23. In 1902 I was working on the unfertilized egg and few attempts were made at fertilization; hence the data are rather incomplete for that year. At first glance my data do not appear very significant, but one readily notices a general tendency for the oviposition to occur near the time of spring tides. This is especially true in those experiments where I found mature sexual products in greatest abundance. For example, in 1902 the best success in fertilization occurred on July 7, two days after new moon; in 1903 the best results obtained for the season were at the time of new moon and the day following, July 23 and 24; in 1907 best results happened on July 26, two days after full moon; and in 1908 at new moon, July 28. The full meaning of these facts, however, is best brought out in Table II. Here I have shown the day on which eggs and sperm were deposited in the laboratory in reference to spring tide. In all I have recorded 38 instances where *Amphitrite* have shed eggs or sperm in an apparently normal manner. Twelve of these were known to be injured, and consequently their data are unreliable. But of the twenty-six uninjured worms twenty-three (88 per cent.) shed their products on or within two days of spring tide; twenty-four worms (92 per cent.) shed products within three days of spring tide; only one worm deposited eggs five days, another seven days, after the period named. Of the injured worms nearly sixty per cent. deposited products within two days of this period, while thirty-three per cent. missed the spring tide by more than four days. This suggests the possibility that, of those worms given as uninjured, perhaps the last two were injured internally. Again, the table shows that more worms laid eggs or sperm on the day of spring tide than on any other day; that the next largest number deposited products on the day following; that practically all sexual products are deposited within three days of spring tide with a tendency to follow rather than to precede this period. That the distribution of these figures is not due to a like distribution of the number of times worms were collected, is shown clearly by comparing them with the figures in the last line of the table.

In order to make a further test of the hypothesis that ripe sexual products are deposited most abundantly at the time of spring tide, on July 17, 1909, the date of the new moon, twenty adult *Amphitrite* were collected. Of this number twenty-five per cent., three females and two males, deposited ripe products, thus giving excellent confirmation to the hypothesis. The question arises how may we account for the periodicity in the time of egg-laying. Observations that have been made upon some other forms will help in our explanation.

Mayer ('02) has described the interesting case of the breeding habits of the Atlantic palolo. This worm "swarms at the surface before sunrise within three days of the day of the last quarter of the moon between June 29 and July 28." "All eggs mature simultaneously at the time of the normal swarm." Later observations ('09) show that the time of swarming is not so definite as at first supposed. "When the last quarter of the moon falls late in July there may be a response to the first quarter as well as to the last quarter." "A dense swarm occurred on July 10, 1908, a fairly dense swarm on July 19." This behavior shows that a particular change of the moon has no direct effect on the time of swarming, as the moon was in first quarter July 6 and in last quarter July 20. Mayer also performed an important experiment to test the effect of tides on the time of swarming. He writes as follows: "Some worms swarmed normally on July 19 out of the rocks which had been maintained in a floating (tideless) live car for the six weeks previous to the swarm." This experiment appears to demonstrate that tides are without direct effect in producing the swarm. But, as Mayer concludes, it "may indicate that the changing pressure due to rise and fall of tide over the reefs is a contributory but *not a necessary* component of the stimulus which calls forth the breeding swarm."

However, it is known that the tide may form a sort of habit in the action of some animals. Gamble and Keeble ('06) have described a small, green, sedentary turbellarian, *Convoluta roscoffensis*, that occurs on the coast of Brittany. "It exhibits a periodic vertical movement whose rhythm is that of the tide." When the tide is out, they come to the surface, forming green patches on the sand. When the tide comes in they retreat below the surface into safer quarters. The remarkable fact is that when removed from the effects of tidal action by being kept in an aquarium, *Convoluta* continues to perform rhythmic movements in time with the tide. "The rhythm is not profoundly impressed upon it; after a day the movements of the patch in the vessel cease to synchronize with those in the open." This illustration suffices to show how an action may arise in relation to the tide without depending directly upon it.

In *Amphitrite* it has been observed that feeding is more continuous and more active as the time of spring tide approaches. At such times the great mass of tentacles radiate from one opening of the tube and actively explore the vicinity for a distance of several inches. Their food consists of small bits of organic matter carried to the mouth through the action of cilia. One result of the feeding activity is seen in the fact that the immature eggs or sperm grow and mature very rapidly during the last few days immediately preceding the sexual period. Undoubtedly, this increased activity of the organism is stimulated by the higher temperature of the sand flats at the time of spring tide. The alternate increase and decrease in the depth of the water, thus altering the pressure, probably has some influence and the food supply at this period is certainly more abundant. We must conclude therefore that *the influence of the tides or moon is entirely secondary. The sexual activity in these worms is closely associated with a similar rhythmical period of greater bodily activity; and this greater bodily vigor of the animal is induced by conditions that depend upon the tides.* Furthermore, the periodic sexual reflex has acquired a sort of physiological basis in the organism, for the worms deposit sexual products normally, when removed from the influence of the tide. Still this reflex has not become a habit in the animal, at least not a strong one, for if a worm does not deposit its products within a few hours after being captured it rarely does so, and then not later than the following day.

II. Method of Egg-Laying.

The eggs of *Amphitrite* break loose from the matrix of the germinal epithelium in early stages of development and complete their growth while floating free in the cœlomic fluid. In a single worm they are usually found in the various stages of development. When first collected, all worms go through with a series of rhythmic movements of the body. When performed in the tube, these movements are evidently for the purpose of aeration, and they are kept up for some hours after the animals are removed from the tube, gradually diminishing in intensity. Each series of movements begins as a contraction near the posterior end of the body and travels forward; a second contraction follows, and frequently a third has begun before the first has disappeared. Between the contractions are wave-like enlargements that serve as moving valves to pump the water slowly through the tube. This pumping action may be demonstrated by placing a recently captured, uninjured worm in a U-shaped glass tube of suitable size. When *Amphitrite* is about to deposit its eggs the movements become more rapid and frequently more violent than usual. Quoting from my notes, the further process is somewhat as follows : "While the worm's body is undergoing the series of slow peristaltic movements, consisting of contraction-waves that begin near the posterior end and travel forward, the eggs ooze out in string-like, sticky masses that are soon scattered by the movements of the body, or by currents of water. The eggs are extruded through five openings in the anterior region of the body, the first opening being on the second segment back of the third pair of gills, or the sixth body segment, not counting the prestomium. Sperm is extruded through similar openings of the same number and location." These openings are nephridiopores that have become specialized as gonaducts, and it should be added that they are laterally placed, lying between the dorsal and ventral chætigerous lobes.

In a previous paper I have mentioned the fact that the eggs are greatly flattened in the polar diameter at the time of extrusion, and that the first polar spindle is in the metaphase. I have also mentioned above the fact that these worms possess the power of separating ripe and unripe eggs in the process of oviposition. How is this accomplished? Some dissections were made in an attempt to answer the question. It was found that this species possesses the same general arrangement of internal organs as that of other *Amphitrite* described by Meyer ('87). "Alle Nephridien der Terebelloiden, sowohl die vorderen als die hinteren, münden im Bereiche desjenigen Korperzonites, welchem sie angehören, einzeln und unabhangig von einander nach aussen ; ihre Wimpertrichter haben eine intersegmental Lage und öffen sich stets in das nächst vorangehende Segment." All the septa in the Terebellidæ are incomplete with one exception ; this one, the diaphragm, is strongly developed and separates the anterior region of the body cavity from the rest. In the species here described the diaphragm is between the fourth and fifth body segments. The external openings of the excretory nephridia open on the third, fourth and fifth segments, but the inner openings are all anterior to the diaphragm. The inner openings of the post-diaphragmatic nephridia are fimbriated membranes, consisting of dorsal and ventral portions in close apposition, covered by cilia ; each of these openings leads into a large membranous sac that is well supplied with blood capillaries and is also ciliated. These nephridia serve as gonaducts, and probably serve also as excretory organs. When egg-laying was first observed, I thought the collapse of the egg in the polar diameter might have a direct relation to the separating process, but there is nothing in the structure of the nephridia to indicate that the eggs undergo a sifting process. Besides the cœlomic corpuscles do not escape and they are smaller than either ripe or unripe eggs.

Somewhat similar phenomena have been observed by Gerould ('06) in *Phascolosoma*. "A few hours before egg-laying occurs, the nephridia become distended with a transparent fluid." "Ova that are ready for maturation, having the spindle of the first polar body in metaphase, are swept from the cœlom into the nephridia by the action of cilia which give rise to strong currents within the nephridium, setting from the nephrostome backward towards its posterior extremity. This is a most interesting process in that both the immature ovocytes, which are present in great numbers in the cœlomic fluid, and the cœlomic corpuscles are excluded from the nephridium, while the fully grown ovocytes are collected there in great numbers." Gerould presents a tentative explanation somewhat as follows. The transparent fluid, he thinks, is sea-water, taken in through the nephridiopore, and he believes that ova in the early stages of maturation probably absorb water while within the nephridium. "If eggs in the earliest stages of maturation show a tendency within the nephridium to absorb sea-water, may it not be assumed that ova at that stage are positively hydrotropic? On this supposition we may explain why such eggs are caught up from the cœlomic currents into the nephrostomal region, and thence carried into the nephridium." This assumption, however, is not to be seriously regarded and is, I believe, incorrect ; at least it is incorrect in the case of *Amphitrite*.

It is rather an easy matter to separate the ripe eggs from the immature ones and from the cœlomic corpuscles by decanting after stirring the mixture in sea-water ; the largest ova and the ripe eggs, those with the first maturation spindle in metaphase, always settle more quickly to the bottom of the dish. The immature eggs then settle, and last of all the cœlomic corpuscles. *It is not an assumption, therefore, to say that the largest ova including those in the early stages of maturation, are more quickly influenced by gravity than the other bodies floating in the cœlomic fluid.* This is the important fact, and is no doubt due to the larger amount of yolk in such eggs.[1]

If, as Gerould supposes, a hydrotropic attraction is necessary to separate these eggs from the other bodies in the cœlomic fluid, then this influence would be useless for the purpose of separation when the contents of the body cavity are emptied in sea-water. For, surrounded by water, the hydrotropic influence would act in all directions and result in equilibrium. Such is not the case. Indeed, Gerould noticed such facts and in appendix B describes how large individuals should be opened in sea-water. "When the female with an abundance of eggs is found, the maturer ovocytes should be allowed to settle to the bottom, whereas the smaller ovocytes and cœlomic corpuscles should be decanted after a few seconds, and before they have had time to sink."

That gravity forms the differential by which the separation takes place is supported by a considerable number of facts. In the course of one period of oviposition, usually extending from one to one and one half hours, the eggs at first deposited are practically all in the metaphase of the first polar spindle ; in the latter part of the period there is always a considerable number, and sometimes a majority, of ova deposited with the germinal vesicle intact. Upon killing a worm that is through egg-laying, one may still find a few scattered eggs in early maturation. If the separation depended upon a tropism, one does not see why it should be so much more complete at one time than another.

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[1] Whether this tendency of large ova to settle quickly is due to a greater specific gravity, or to a greater mass in proportion to the amount of surface offering resistance, the end result is the same. This question must be decided by further investigation. In this paper we shall speak of the large ova as though they had a greater specific gravity than the smaller cœlomic bodies.

Again, the nephridia are not extraordinarily large, and each must be refilled many times in the course of one oviposition where thousands of eggs are deposited. Nor do the nephridia ever seem to be entirely filled with eggs; at least such is the case in worms that are killed almost instantly with hot sublimate acetic while in the act of oviposition. The meaning of this will appear in the explanation. It is also manifestly clear that the movements of the body referred to before, cause the body contents to move forward toward the diaphragm. As a rule, each effective contraction-wave stops a short distance posterior to the nephridia and holds for a moment the compressed cœlomic contents in the much distended anterior portion of the body. During the final part of a contraction-wave, a stream of eggs oozes out a short distance from each nephridiopore posterior to the diaphragm. The contraction movements, therefore, are necessary for the expulsion of the eggs.

The explanation which I believe accords best with all the known facts is as follows: First, it should be remembered that the nephridial sacs always occupy a lower position with reference to gravity than the nephrostome, whether the worm lies in a horizontal or vertical direction. We may then think of the nephridia as settling basins in which the heavier products are drawn off from the bottom after a certain amount has accumulated. Ciliary action undoubtedly would prevent lighter objects, such as the cœlomic corpuscles and the unripe eggs from settling in the basin; and when a sufficient quantity of the ripe eggs (heavier objects) have accumulated, pressure from the strong contraction-wave forces them through the nephridiopore. The separating process probably takes place during relaxation, between contraction waves. Owing to the opacity of the worm's body, it is impossible to actually observe this process, but all of the facts point to this simple explanation.

Summary.

1. In *Amphritite ornata* Verrill, the egg-laying reflex is closely associated with the time of spring tide; the height of any given period of egg-laying always occurs within two days of the time of new or full moon. Periods of oviposition occur in June, July and August.

2. The moon does not have any direct influence in producing the period of sexual activity. It is probable that the tide also has little, if any, direct effect on the process.

3. At spring tide, the worms feed more actively, the food supply is more abundant and the sand flats have a higher temperature. As this period approaches we also find a more rapid growth and development of immature eggs and sperm. Therefore, the period of sexual activity is closely associated with a synchronous period of greater bodily activity, and this greater vigor of the animal is induced by conditions that depend upon the tide. In this way we may explain how oviposition in *Amphritite* has become a sort of reflex habit associated with the time of spring tide.

4. When a worm is sexually mature, the cœlomic fluid contains cœlomic corpuscles and eggs in various stages of development. At oviposition the worm extrudes ripe eggs, and toward the end of the process some of the immature ones, but always retains the much smaller cœlomic corpuscles.

5. Since the mature eggs sink faster in sea-water than the smaller immature ones, and all eggs sink faster than the cœlomic corpuscles, it is believed that the larger eggs have a greater density than the other bodies in the cœlomic fluid; and it is entirely probable that the apparent selection of ripe eggs and the rejection of immature ones is due to the different effects produced by nephridial currents upon bodies of apparently different densities.

6. The position of the nephridial sacs, and the arrangement of cilia on the nephrostomes and within the sacs, is such that we may regard the nephridia as a set of settling basins in which the separation takes place. Contractions of the worm's body then aid in expelling the ripe eggs from the nephridial sacs.

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ON THE USE OF MAGNESIUM IN STUPEFYING MARINE ANIMALS.

ALFRED G. MAYER.

It is well known that Tullberg, 1892, discovered that an excess of magnesium added to sea-water causes anesthesia in marine animals, thus permitting them to be killed in an expanded state.

During the course of some physiological experiments carried out at the Marine Laboratory of the Carnegie Institution of Washington at Tortugas, Florida, I found that marine animals can be anesthetized much more rapidly and completely than by Tullberg's method if we simply place them in a pure aqueous solution of $MgSO_4$ or $MgCl_2$ of three eighths molecular concentration. They then subside into complete relaxation without initial stimulation, and after remaining for an hour or two in the solution they may be killed in any manner whatsoever without becoming distorted through contraction. Some distortion is often produced in Tullberg's process, due to the calcium and sodium of the sea-water, but in a pure aqueous solution of magnesium the relaxation of the muscles is complete. This method has been tried upon scyphomedusæ, ctenophoræ, actinians, annelids, nemertians, phascolosoma, and nudibranchs with marked success, and appears to be especially suitable for the stupefying of highly sensitive and contractile marine animals which become hopelessly distorted if killed by ordinary methods.

It is interesting to observe however that while magnesium is the most potent anesthetic for the neuro-muscular system it is the most powerful stimulant among the ions of sea-water or of blood-salts for the movement of cilia. Indeed I find that the ions of Na, Mg, K and Ca affect cilia in a manner the exact *opposite* of their effect upon muscles and nerves. Thus Na is the most powerful neuro-muscular stimulant, and the most pronounced inhibitor for the movement of cilia. Mg is the greatest inhibitor for nerves and muscles and the strongest stimulant for the movement of cilia. A weak concentration of K at first excites and then depresses the neuro-muscular system, and at first subdues and afterwards stimulates the movement of cilia. Ca is a depressant for nerves and muscles but a weak stimulant for cilia. NH_4Cl is a primary stimulant for muscles but soon produces depression, while upon cilia its effect is the reverse, a primary cessation of movement being followed by recovery. The CO_2 ion inhibits muscular activity, while in weak concentration it produces a primary depression of cilia followed by a recovery of movement.

REGENERATION IN FUNDULUS AND ITS RELATION TO THE SIZE OF THE FISH.

G. G. SCOTT.

Przibram ('09) in his treatise on regeneration has reviewed the work bearing on the relation between the age of the animal and the ability to regenerate. The general conclusion is in the form of a law — "Die Regenerationsfahigkeit nimmt mit zunehmenden Alter eines Tierexemplares ab —." In the BIOLOGICAL BULLETIN ('07) the author came to the conclusion that the regeneration of the caudal fin of *Fundulus heteroclitus* was greater in the shorter than in the fishes of medium length and greater in these than in the longest fishes.

That conclusion was reached by comparing the average specific regeneration of the various groups. The specific regeneration is obtained by dividing the actual amount regenerated by the length of the animal, giving as a result the percentage amount regenerated by that specimen. This term was introduced by Zeleny. Now if we take any number and divide it successively by a series of numbers each greater than the preceding we will obtain a series of quotients each successively smaller and smaller. On referring to the experiments in his former paper the author found that the actual amounts of regeneration differed but little in the various specimens used, while the length of the animals increased, so that the case is as stated above — *i. e.*, that the greater specific regeneration in the shorter fishes is due largely to the fact that the divisor in case of short body length is smaller than the divisor in case of long body length, while the dividend (the actual amount regenerated) is about the same, so that naturally we should get smaller and smaller quotients, which in this case would be specific regeneration. This is seen when we refer to the former paper.

To test the matter further the writer repeated the experiment during the summer of 1908 using a larger number of fishes. The caudal fin of each specimen was removed at the same relative level on August 4 and the fish were placed in running sea-

TABLE I.

Fundulus heteroclitus.

	Length.	Amount Regenerated.	Specific Regeneration.		Length.	Amount Regenerated.	Specific Regeneration.
1	4.57 cm.	.57 cm.	.1247	55	6.80 cm.	.70 cm.	.1029
2	4.68	.54	.1154	56	6.81	.53	.0778
3	4.79	.58	.1210	57	6.86	.64	.0933
4	4.87	.66	.1355	58	6.89	.59	.0856
5	4.96	.65	.1311	59	6.90	.65	.0942
6	4.97	.60	.1201	60	6.94	.59	.0850
7	5.10	.54	.1058	61	6.96	.64	.0919
8	5.12	.59	.1152	62	6.97	.66	.0946
9	5.16	.58	.1124	63	7.10	.62	.0873
10	5.17	.54	.1044	64	7.12	.52	.0730
11	5.28	.60	.1117	65	7.24	.68	.0939
12	5.30	.61	.1151	66	7.26	.80	.1102
13	5.38	.64	.1187	67	7.29	.67	.0905
14	5.40	.60	.1111	68	7.29	.63	.0864
15	5.42	.54	.0996	69	7.38	.58	.0786
16	5.43	.64	.1178	70	7.39	.70	.0947
17	5.44	.61	.1122	71	7.40	.56	.0757
18	5.44	.53	.0974	72	7.44	.69	.0927
19	5.49	.65	.1184	73	7.45	.53	.0711
20	5.52	.69	.1250	74	7.48	.56	.0748
21	5.63	.66	.1172	75	7.49	.50	.0667
22	5.64	.61	.1081	76	7.50	.54	.0720
23	5.69	.59	.1031	77	7.50	.49	.0653
24	5.70	.78	.1368	78	7.52	.50	.0665
25	5.70	.66	.1158	79	7.53	.60	.0975
26	5.73	.68	.1193	80	7.56	.58	.0766
27	5.79	.60	.1036	81	7.60	.70	.0921
28	5.80	.58	.1000	82	7.62	.73	.0945
29	5.90	.60	.1002	83	7.69	.54	.0702
30	5.93	.65	.1096	84	7.74	.54	.0967
31	5.96	.57	.0956	85	7.77	.62	.0798
32	5.96	.64	.1074	86	7.87	.60	.0763
33	5.99	.78	.1302	87	7.88	.54	.0685
34	6.04	.67	.1109	88	7.92	.56	.0701
35	6.20	.62	.1000	89	7.94	.55	.0683
36	6.26	.67	.1070	90	8.00	.47	.0587
37	6.40	.70	.1094	91	8.00	.65	.0813
38	6.46	.50	.0774	92	8.07	.59	.0731
39	6.48	.68	.1049	93	8.10	.62	.0765
40	6.49	.52	.0801	94	8.23	.54	.0656
41	6.50	.60	.0923	95	8.23	.52	.0632
42	6.50	.60	.0908	96	8.25	.55	.0666
43	6.50	.57	.0877	97	8.27	.57	.0689
44	6.53	.53	.0812	98	8.27	.45	.0544
45	6.53	.54	.0827	99	8.37	.37	.0442
46	6.55	.63	.0962	100	8.38	.65	.0775
47	6.62	.47	.0710	101	8.38	.56	.0668
48	6.63	.60	.0815	102	8.39	.60	.0671
49	6.65	.63	.0947	103	8.40	.65	.0774
50	6.69	.63	.0942	104	8.56	.64	.0746
51	6.72	.54	.0803	105	8.69	.50	.0575
52	6.73	.63	.0937	106	8.91	.56	.0628
53	6.75	.42	.0622	107	9.00	.53	.0590
54	6.76	.64	.0974	108	9.73	.55	.0656

water in the Biological Laboratory of the U. S. Bureau of Fisheries at Woods Hole, Mass. I am indebted to Dr. Francis B. Sumner, the director of the laboratory, and to the commissioner, the Hon. George M. Bowers, for the facilities extended. The fishes were fed regularly until September 5, a period of a month, during which time new caudal fin tissue regenerated from the cut surface of each fin. The fishes were then removed, carefully preserved and later measured. There were alive, on September 5, 108 *Fundulus heteroclitus* and 50 *Fundulus majalis*. Table I. shows the length, the amount regenerated and the specific regeneration of each *Fundulus heteroclitus*. Following this is Table II. which shows the same with regard to the *Fundulus majalis* used.

Table II.

Fundulus majalis.

	Length.	Amount Regenerated.	Specific Regeneration.		Length.	Amount Regenerated.	Specific Reneneration.
1	6.79 cm.	.60 cm.	.0899	26	8.08 cm.	.47 cm.	.0582
2	6.95	.58	.0835	27	8.13	.39	.0480
3	7.13	.53	.0743	28	8.18	.60	.0733
4	7.32	.54	.0738	29	8.20	.50	.0549
5	7.40	.55	.0743	30	8.23	.40	.0485
6	7.40	.45	.0601	31	8.24	.63	.0764
7	7.45	.55	.0768	32	8.26	.49	.0593
8	7.49	.55	.0734	33	8.26	.52	.0629
9	7.49	.55	.0734	34	8.33	.53	.0636
10	7.50	.49	.0658	35	8.45	.49	.0580
11	7.50	.55	.0733	36	8.50	.50	.0419
12	7.55	.48	.0635	37	8.57	.53	.0618
13	7.55	.40	.0530	38	8.60	.50	.0580
14	7.60	.53	.0698	39	8.60	.42	.0488
15	7.61	.55	.0722	40	8.67	.44	.0588
16	7.67	.53	.0691	41	8.72	.57	.0654
17	7.77	.55	.0708	42	8.75	.39	.0466
18	7.80	.55	.0705	43	8.80	.38	.0432
19	7.84	.35	.0446	44	8.96	.40	.0451
20	7.88	.50	.0634	45	8.91	.46	.0516
21	7.89	.45	.0570	46	9.17	.50	.0545
22	7.93	.59	.0744	47	9.20	.60	.0652
23	7.93	.55	.0693	48	9.60	.30	.0318
24	7.95	.56	.0704	49	9.73	.42	.0432
25	8.00	.40	.0500	50	10.50	.49	.0466

On examining the specific regeneration in the case of *Fundulus heteroclitus* there is seen to be a gradual fall in percentage from the shorter to the longer fishes. To make this more plain we can divide the fishes into groups ranging from the shorter to the longer, differing in length from the adjacent groups by one half centimeter, find the average specific regeneration of each group and express the results as follows :

Group.	Range in Length.	Number of Specimens.	Average Specific Regeneration.
1	4.5– 5.0 cm.	6	.1243
2	5.0 5.5	13	.1108
3	5.5 6.0	14	.1123
4	6.0 6.5	7	.0885
5	6.5 7.0	22	.0878
6	7.0 7.5	13	.0843
7	7.5 8.0	14	.0762
8	8.0 8.5	14	.0672
9	8.5 9.0	3	.0649
10	9.0 9.5	1	.0590
11	9.5 10.0	1	.0656

From this arrangement it would appear that the shortest regenerated 12 per cent. of their length while the longest regenerated 6 + per cent. and that on the whole as the length of the fishes increases the percentage regeneration decreases. On the other hand if we run over the column giving the actual amounts of regeneration we see that in a general way they are much the same for the longer as well as for the shorter fishes. This becomes evident when we ascertain the average actual regeneration for each of the groups mentioned above. This can be arranged as follows :

Group	1	2	3	4	5	6	7	8	9	10	11
Av. Reg., cm.	.60	.59	.65	.62	.59	.62	.58	.56	.57	.53	.55

On examining again the table giving the actual regeneration of each specimen we find some that may be regarded as extreme variants. These are nos. 24 and 33, in which the regeneration is .78 cm., no. 66 with a regeneration of .80 cm. and no. 99 in which the regeneration is .37 cm.

If we make allowance for these and obtain new averages for the groups in which they occur our series will be as follows. We will disregard the last two groups on account of the small numbers of specimens.

Group	1	2	3	4	5	6	7	8	9
No. of Sp.	6	13	12	7	22	12	14	13	3
Av. Reg., cm.	.60	.59	.63	.62	.60	.60	.58	.57	.57

If we compare the average regeneration of groups 1 and 2, representing 19 fishes from 4.5 cm. to 5.5 cm. in length, with the average regeneration of groups 8 and 9, representing 16 fishes from 8.0 cm. to 9.0 cm. in length, we find that there is a difference of but .025 cm. Again, if we compare the average regeneration of groups 2 and 3, representing 25 short fishes, with that of groups 7 and 8, representing 27 longer fishes, we find a difference of but .035 cm. The relation of the specific regeneration to the actual regeneration is represented by the following diagram (Fig. 1) in which the base line represents the length of the fishes — the upper curve *A–B* was formed by joining the points representing the specific regeneration in the various groups and therefore represents the relation of the specific regeneration to length. The lower curve, *C–D*, was formed by drawing a line through the points representing the actual regeneration in the various groups and therefore represents the relation of the actual regeneration to length. It is to be noted that while on the whole the curve representing relative specific regeneration falls, at the same time the curve representing the actual regeneration remains almost parallel with the base line although it will also be noted that there is an indication of a slight decrease in regeneration. There is a strong indication, however, that the longer fishes have regenerated almost as much tissue as the shorter in the same length of time.

But what is the condition in the case of the *Fundulus majalis* the results of the experiment with which are given in Table II. On arranging the results in a way similar to that used in the case of *Fundulus heteroclitus* we have the following:

Group.	Range in Length.	Number of Specimens.	Amount Regenerated.	Average Specific Regeneration.
1	6.5– 7.0 cm.	2	.59 cm.	.0867
2	7.0 7.5	7	.52	.0709
3	7.5 8.0	15	.51	.0658
4	8.0 8.5	11	.49	.0594
5	8.5 9.0	10	.46	.0521
6	9.0 9.5	2	.55	.0598
7	9.5 10.0	2	.36	.0373
8	10.0 10.5	1	.49	.0466

Here again it will be observed that there is a gradual decrease in the specific regeneration. The number of specimens in groups 1, 6, 7 and 8 is so small that too much value must not be placed on them. Although the total number of specimens is smaller in

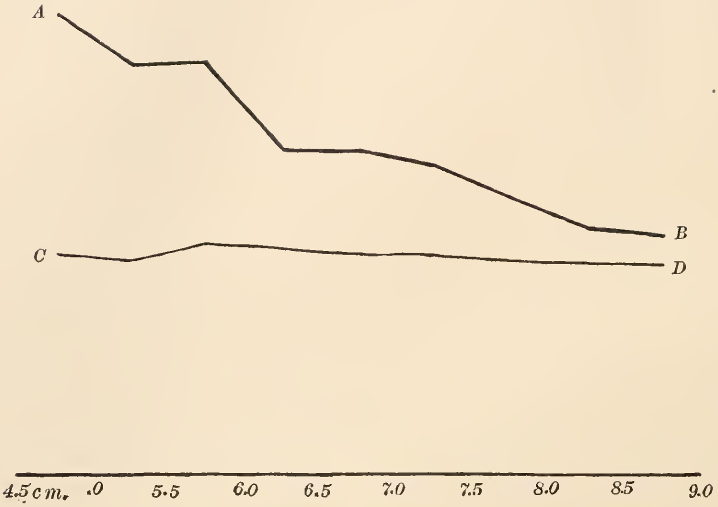

Fig. 1

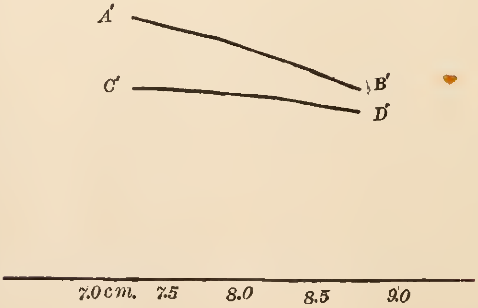

Fig. 2

this case, yet it is seen that the average regeneration is about the same, although here again is observed the slight decrease. The average regeneration of groups 2 and 3, consisting of 22 fishes between 7.0 and 8.0 cm. in length, is .515 cm., while that of groups 4 and 5, consisting of 21 fishes between 8.0 and 9.0 cm. in length, is .475 cm., showing a difference of .04 cm. between the two groups. These results are shown graphically in Fig. 2, in which the curve *A′–B′* shows the relation of the specific regeneration to length, while the curve *C′–D′* shows the relation of the actual amount regenerated to the length. Turning to the results recorded in the former paper we find that they are less satisfactory on account of the smaller numbers. But arranging the results recorded there in a manner similar to that used here we find that on the whole the longer fishes regenerate almost as much as the shorter, although the indication of the slight diminution of actual regeneration with age is not so clear, which may be due to the fact that there are less specimens in the various groups.

The general result seems to be then that the amount of regeneration in the period of time referred to appears to be about the same in fishes of all lengths, although there is an indication of a slight decrease in the case of increasing body length.

Professor Zeleny in a paper to be published shortly (Oct., '09) in the *Journal of Experimental Zoölogy* has found with respect to the regeneration of the tail of the salamander, *Amblystoma jeffersonianum* that rate of regeneration was as follows:

In a series	22.6	mm. long	there was a	regeneration of	.39	mm. per day.
"	26.5	"	"	"	.41	"
"	26.8	"	"	"	.39	"
"	51.6	"	"	"	.32	"
"	54.1	"	"	"	.27	"

These results agree with those described above. There is a maintenance of a high degree of regenerative power in the older specimens. There is also an indication of a slight decline such as we found in the case of the fishes used.

In carrying on an extensive series of observations on the age of fishes Fulton ('06) estimated the age by finding the different modal lengths that occurred in a large number of specimens of a given species. Though the numbers used in this experiment are far too few to enable one to estimate the exact age of the various groups yet reasoning as Fulton did we can at least say that the longer fishes are the older. Stating the matter in terms of age the above experiments appear to indicate that within the limits of age as represented in the series the actual regeneration is the same or slightly decreases with age. It may be objected that the longer (or older) fishes regenerated more in mass than the smaller and that therefore should we determine the mass for each specimen we might find that the larger regenerated more than the smaller. To answer this objection let us suppose that Figs. 3 and 4 represent respectively the caudal fins of one of the shorter and one of the longer fishes. The straight vertical line in each case represents the place of amputation. The dotted vertical line represents the outer limit of new regenerating tissue at the end of a month. According to our results the perpendicular distance from the line *a–c* to the line *b–d* is nearly the same as that from *e–f* to *g–h*. When the amputation was made it left cells exposed along the surfaces represented by the lines *a–c* and *e–f*. In a short time the regeneration of new tissue began. In Wilson's "Cell," 00, page 388, we find that "measurements of cells from the epidermis, the kidney, the liver, the alimentary epithelium and other tissues show that they are on the whole as large in the dwarfs as in the giants. The body size depends on the total number of cells rather than on their size individually considered and the same appears to be the case in plants."

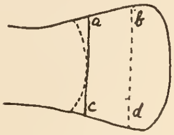

Fig. 3.

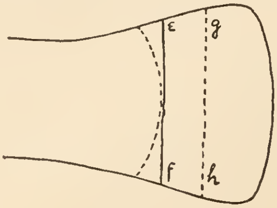

Fig. 4.

So we can conclude that the cells from which new tissue regenerates along the surface represented by the line *a–c* are of the same size as those represented by the line *e–f*. It is apparent that the tissue along the direction of *a–b* has been formed from cells at *a*, and that the tissue along the line *c–d* from cells at *c*.

So for every point in the line *a–c* the tissue opposite every point in this line has been formed from cells in the line *a–c* outward in a direction perpendicular to that line. And so for the newly formed tissue in case of Fig. 4, the tissue opposite every point in the line *e–f* has been formed from cells in the line *e–f* outward in a direction perpendicular to the line *e–f*. Of course in the case of larger fishes (Fig. 4) it is apparent that a greater mass of tissue is formed than is true of smaller fishes (Fig. 3). But is this not due to the fact that there are more cells along the surface represented by the line *e–f* from which regeneration can proceed than there are similar cells along the surface indicated by the line *a–c*. The solution of the problem as to the relation between age and rate of regeneration depends upon the results of measurements made in this manner. And by the amount of tissue regenerated has been and will be meant the length of newly formed tissue measured outward from the line of amputation.

If the cells in the shorter and longer (younger and older) fishes have the same degree of activity then the same amount of tissue ought to be formed outward in a line perpendicular to the cut surface in the same length of time. This is seen to be practically the condition in the case of the experiments presented here. What explanation can be offered for this? Jordan, '05, speaking of the growth of fishes says that "Most of them grow as long as they live and apparently live until they fall victims of some stronger species." Fulton, '06, gives the following law with regard to the growth of fishes: "Fishes approximately double their size and increase their weight about eight times after they have reached sexual maturity." Probably most of the fishes used in these experiments had reached sexual maturity. May we not then correlate this maintenance of a high degree of regenerative power with the continuous growth throughout the life of the members of this group.

But there appears to be a slight decrease in the amount regenerated as the age increases. In computing the difference of the means and the probable differences between adjacent groups of the different series it was found in most cases that at least twice the probable difference was less than the difference of the means and only in case of extreme groups was the difference of the means less than three times the probable difference. This indicates that in adjacent groups the amount of regeneration is very nearly the same, but that on the whole there is a tendency toward a decrease, as we pass from the younger to the older. We can sum up our results in this statement: The power to regenerate new tissue remains remarkably active throughout life but as the fish grows older this power gradually diminishes, which after all is in agreement with Przibram's law. This also is in harmony with the view that regeneration is a growth phenomenon as shown above. Minot, '90, says: "There is a progressive loss of vitality going on probably throughout the entire period of life." Kellicott, '08, found that the organs of the dog-fish which have to do with nutrition and therefore the growth of the organism increase by constantly decreasing increments with increasing size of the animal. The slight decrease in regenerative power which we have noted above parallels then this slowly decreasing rate of growth characteristic of all animals, but which decrease is less evident in those forms having indeterminate growth such as fishes.

Kellicott, in the paper referred to above, found that while the organs of nutrition have increments of growth successively smaller, yet the "muscles and supporting tissues seem to outgrow the brain and viscera leading ultimately to a loss of physiological balance within the organism." But this decrease in growth of the organs of nutrition does not come on suddenly but gradually, so that it must eventually cause a gradual retardation of the growth of the entire animal. If this be true and if the rate of regeneration is affected by the rate of growth, then we should expect to find evidences of gradual diminution in the rate of regeneration. We have seen an indication of this in the above experiments. Finally, it has been noted that mammals and birds have little power of regeneration as compared with amphibia and fishes. May not this be possibly correlated with the different types of growth which these groups possess.

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SOME LIGHT REACTIONS OF THE MEDUSA GONIONEMUS.

L. MURBACH.

In the following notes it is my purpose to record some observations I have made on the behavior of *Gonionemus*[1] to light, after the experiments made a few summers ago on the reactions of its subumbral papillæ to light, and to include in the discussion points on which other observers are not agreed. I shall refer only to these publications.

After observing the behavior of *Gonionemus* for a considerable time the following brief statements will be found to hold concerning their habits. As darkness approaches the medusæ become restless in their native haunts where they are either lying inverted on the bottom, the apex of the bell being heavier, or clinging to plants and other submerged objects. Although all the tentacles in this species have adhesive pads near the free end, yet the animals attach by only a few, the remaining tentacles being spread out in all directions ready to catch their passing prey. When still daylight above the water it is becoming dusk, we may say, at their depth, and among the plants from one half to several meters deep. Within an hour after dark the eggs and sperm are deposited and their intermingling in the sea water increases many times their chance of development. Thus their locomotion in early evening is of great value as then more eggs will be fertilized. The dehiscence of the eggs and sperm has been shown to be due to the diminution or withdrawal of light, and seems rather direct evidence of an external stimulus causing a physiological change. For considerable time after the dehiscence of the sex products the animals lie expanded on the bottom or suspended, and may remain so as long as the light conditions are the same, full darkness having set in. It is probable that much of their prey is captured at this time. As the light grows brighter above, they again move about until they get into weaker light which generally takes them into lower regions or into shaded places, in weak or subdued light. Whenever disturbed, especially by change of light intensity, this medusa swims about in all directions, stopping to float down with expanded tentacles and inverted bell. Again it displays a most striking behavior; it swims almost vertically to the surface of the water by pulsations of the muscular bell, turning over at the surface, expanding and gracefully floating down. Much has been made of this particular behavior by nearly all those who have observed *Gonionemus* for some time. It has been referred to as "fishing" and "surface reaction," the latter term being more satisfactory because less anthropomorphic. The main feature of this behavior is the swimming up toward the surface and floating passively down again after turning over at the surface. This may be renewed as long as the same stimulus acts, or until the condition of the medusa changes so that it no longer responds.

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[1] There would seem to be no need of stating that the Woods Hole species is the one under consideration. The experiments on which these notes are based were made at the Marine Biological Laboratory, at intervals between other work in the summer. I gladly acknowledge the courtesy of the Director in continuing to grant the necessary facilities. It will be found that some points differ from a report made on this subject in the winter before the Michigan Academy of Science (Annual Report, 1909) as I have been able to make additional experiments this season.

From the foregoing it will be readily understood that these medusæ are sensitive to light influences, getting away from strong light — especially during the earlier part of the day and afternoon — later again moving up toward the fading light. In general, Yerkes,[2] who has made most observations on the reactions of *Gonionemus*, says: "Clearly, the animals are attuned, so to speak, to a certain range of light intensity, and are negative in their reactions to higher intensities." Any marked change in this intensity causes locomotion which under natural conditions brings the medusa into the light suitable for its life processes. Whether this optimum intensity is constant or changes with the activities of the animal has not been determined, but ordinary observation indicates that its range is not very wide; it may be called weak light.

On account of the influence of light on these medusæ they collect in the weaker light of an ordinary glass aquarium placed before a window (cf. Fig. 1). If the light is still stronger than their normal they will continue in their attempt to get farther away. When the aquarium is evenly lighted with subdued light the medusæ are evenly distributed and generally at rest. This may be brought about by darkening the window, and the room if necessary, until the light is uniformly weak. If, now, the side of the aquarium away from the window is darkened still more, then the medusæ are again set into activity, moving about until, after some minutes, they will be found collected in the side of the aquarium near the window.[3] In nature there is probably no collecting in groups, as they are not confined.

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[2] *Amer. Jour. Physiol.*, 1903, Vol. IX., page 286.

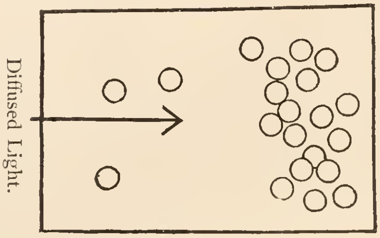

FIG. 1. Top View.

DIRECTED MOVEMENTS.

The main question in my mind has always been whether these medusæ are really directed by light, or properly oriented, even if they could keep the course, *i. e.*, continue oriented or directed. My own experiments have led me to believe that the movements of *Gonionemus* in response to light are not so definitely directed by light as has heretofore been held, and that quantitative differences near their optimum constitute the natural light stimulus. In regard to directed movement, Yerkes[4] says: "It is impossible because of the form of the medusa and its mode of locomotion, that the direction of its movements be as accurately determined by light stimulation as are those of . . . other animals whose structure permits of more accurate orientation in reference to the source of light." While no doubt in a measure true, to me it seems that this statement is not wholly borne out by the fact that it can swim in nearly straight lines when coming to the surface, in the so-called surface reaction. In fact, the only stimulus to which it seems to respond in pretty straight lines is gravity, though the question of the influence of light in the "surface reaction" is difficult to decide.[5] Yerkes's statement[6] "that the direction of its movement is definitely determined by light" is based on analogy and not on experiment. In this paper[7] such expressions as "movement toward the source of light," and "strong light . . . soon repels the animals"; again, "an animal passes from the shadow into the sunlight" (page 305), I do not take to mean that the animals swam directly toward or from the light. However, if this is meant, the strongest argument in favor of the medusæ's swimming in a direct line not vertical is (page 282) where a medusa inhibited by strong light, starting up again, "usually turns in such a way as to move back into the shaded region." Morse[8] reports a similar experiment differing in that he observed the movement of medusæ in the sunlit half of the dish, saying, "the medusæ begin to swim in all directions." With this my observations agree. Indeed, in Yerkes's answer to this criticism of Morse's, having repeated the experiment, he says: "I found that when the animals swam so far into the sunlit region before turning over that they were entirely in the sunlight when they came to rest on the bottom of the dish, they moved away from the region of shadow about as often as toward it."[9] In this, then, he agrees with Morse's contention that the medusæ do not turn directly toward the shadow and swim into it more often than away from it. In fact he adds (page 462) "with the light perpendicular to the bottom of the vessel I obtained the same results as Mr. Morse. There was no evidence of the directive influence of light."[10] But Yerkes (page 461) also points out that his inference from his original experiment was correct for all medusæ falling on the border between sunlight and shade so that "part of the body is in the shadow."

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[3] Because of the observation above I cannot agree with Morse that *Gonionemus* is not positively phototactic.

[4] *Amer. Jour. Physiol.*, 1902, Vol. VI., p. 448.

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[5] This "surface reaction" has been observed in three species of the genus, geographically far enough removed from each other that it seems to indicate an ancestral character.

[6] *Amer. Jour. Physiol.*, 1903, Vol. IX., page 285.

[7] *Amer. Jour. Physiol.*, 1903, Vol. IX., p. 284.

[8] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., p. 454.

[9] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., p. 461.

[10] Page 462 Yerkes suggests that the contradiction between himself and Morse in regard to oblique light might disappear if Morse's meaning of the term were more fully explained. Turning to Morse's experiment we find (pp. 453–454) that he used

Does Light Orient *Gonionemus?*

Although *Gonionemus* does not move parallel to the direction of stronger light it has been held that its movements are directed by stronger light and that it thus gets from an unfavorable to a favorable light place by direct responses or movements suited to this purpose. Yerkes says:[11] "This is apparently accomplished by the more forceful and earlier contraction of that side of the bell farthest away from the shadow." In the case of other stimuli (tactual and electrical) he had demonstrated this but not in the case of light. Though, page 285, we read: "Observation indicates that the side of the organism which is exposed to the most intense light contracts first and most strongly thus forcing the bell over," yet there is scarcely the weight of proof in this observation. In a later paper Yerkes[12] says: ". . . brilliant illumination of one side of the bell . . . brings about movement toward the region of lower illumination." This is based on an experiment of throwing sunlight on part of a medusa. He gives 66 per cent. as turning toward the region of lower illumination.

Morse[13] restates the same explanation of the turning mechanism, and from the experiment of mutilating one side of the medusa shows that by the resulting one-sided contraction circle swimming is induced. In a later publication[14] he has shown that light has the same effect, *i. e.*, to turn the animal. Half of a medusa in the dark was illuminated by a vertical beam of light. This caused ". . . the medusa to swim vertically upward, and it was only after it had pulsated three or four times that its path veered from the perpendicular. The result of one hundred trials,

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only sunlight falling perpendicularly, and in another experiment in which he used oblique light it had no reference to Yerkes's former experiment. More medusæ would fall in oblique light with bodies partly in the shadow than in vertical.

[11] *Amer. Jour. Physiol.*, 1903, Vol. IX., p. 284.

[12] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., p. 461.

[13] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., p. 451.

[14] *Amer. Nat.*, 1907, Vol. XLI., p. 683.

upon different individuals in the main" gave 70 per cent. in favor of the view "that light has a directly orienting effect." In considering this experiment we should know how many different individuals were used, and should bear in mind that the time limit for response was from five seconds to three minutes, and that the turning began to show only after several pulsations. This would seem to be different from Yerkes's view which is that the medusæ turn directly on stimulation by strong light.

As my experiments were made before the above results were published, and they favor the view of Yerkes that strong light turns the animal immediately they may be added here. The first test was made by using a horizontal band of sunlight as wide as the aquarium, 1 cm. deep, and a little distance — 5 cm. — from the bottom. Darkening the aquarium momentarily was the means of starting the medusæ swimming up toward the band of light. Usually five or more medusæ were used to begin the experiment. Forty-three per cent. were turned back by the band of light, away from the source; 33 per cent. turned toward the source; and 24 per cent. swam straight through the band of sunlight.[15] Now it was thought that an oblique band of sunlight (similar to Fig. 3) would be more decisive, as one side of the up-swimming medusa would be stimulated, not only more strongly, but in advance of the other. In this case 50 per cent. of the medusæ turned away from the direction of the sunlight and 41 per cent. toward it; 9 per cent. did not respond. A relaxed medusa, allowed to float bell downward, showed a more striking result on touching the oblique band of sunlight. It turned, and after swimming upward a few strokes, floated down, only to do the same on striking the band; the next time it floated through and on emerging below turned in the opposite direction, again away from the band of sunlight, but it continued in a circle which again carried it up through the sunlight.

There is enough difference in methods employed in our experiments to leave no doubt that the medusæ do turn at a sudden transition into strong light, especially when they are in very weak light. Morse obtained his result with a limited number of resting or moving medusæ. From the conditions of his experiment we may infer that in some cases he did not get a response in less than three minutes. In my own experiments I rejected any reactions that did not take place within a minute after the stimulus was applied. As to the direction of turning into or away from sunlight there is not so great uniformity. Had I taken a few cases like the one of the relaxed medusa above, I would have gotten high percentages. As it is, I have a large enough result (56 per cent.) to conclude with others that *Gonionemus* turns away from strong light. But on carefully observing this turning it is evident that not in many cases do the medusæ turn in such a way that their continued swimming would take them parallel with the light direction. Not infrequently they turn back into the strong light, as has also been observed by others,[15] or are not turned far enough. Is not Morse's ingenious explanation, given below, also a tacit admission that the mere turning by strong light does not cause a medusa to move parallel to the light direction? This turning, therefore, cannot be considered true orientation, and would not lead to swimming directly away from the source of light. But since it has been shown to be a response to strong light, the question remains as to its use to the organism. In nature the medusæ are probably exposed to sunlight only when they are disturbed and swim to the surface, or when the location of one is exposed as the sun's rays come in a different direction. If now the medusæ were to be oriented and swim away from the light it would take them downward, as Morse[16] has already pointed out. It is to be noticed also that both in nature and in aquaria, the bottom prevents this, and in a measure compels swimming toward the region of lesser illumination if the turning by light has been anywhere within half a sphere. It will be seen that this behavior, repeated, even if no further directed movement takes place, will be helpful in temporary escape from strong sunlight.

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[15] I am compelled to agree with Morse that the collecting of this medusa in strong sunlight is not a normal reaction, but rather due to previous excessive stimulation of some kind.

Ways of Getting into Optimum Light.

If I have succeeded, so far, in making my position understood the question will naturally arise, How do the medusæ get into favorable light areas? Although Yerkes does not hold it so, I believe he has indicated one way in his observation:[17] "When an individual in swimming about chances to cross from the sunlit region into the shadow, it very quickly ceases swimming and sinks to the bottom." In another connection but bearing out the same point, Morse[18] has this to say — "being in motion almost incessantly, and swimming in all directions, it is obvious that sooner or later they will enter the dark area. Once having entered this area, the stimulating effect of sunlight being cut off, they remain as in a trap." Even if we do not hold that only light stimulates, or that optimum light acts like a trap the explanation may hold.

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[16] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., p. 455.

More recently Morse[19] has described a way in which *Gonionemus* may get from a location that is unsuitable with respect to light to a more suitable one. A medusa was placed in the end of an aquarium through which the sunlight was reflected horizontally. The medusa swam to the surface in characteristic fashion, each time bending its upward course a little farther from the vertical, and therefore away from the source of light. Thus ultimately it got to the farther side of the aquarium, into weaker light. The promising feature of this explanation is that it is based on the peculiar habit of the animal — swimming to the surface when disturbed. The other case that Morse records of a "strong swimmer" moving directly toward the less illuminated end of the aquarium I should consider an exception, as in repeating the experiment I have observed that some medusæ move almost directly to the lighter end after proceeding, by stages, to the darker end. In my trials I have found that about 25 per cent. get to the farther end of the aquarium by the method indicated, but instead of its being a regular method of progression it seemed to me to be characterized by irregularity. This may be due to the fact that Morse seems to have worked with few individuals, whereas I placed a number in the aquarium at the beginning of the experiment and added to these as the experiment progressed, so as to get results representative of more than individual behavior.[20] While the majority of the medusæ in the experiment finally reached the less illuminated end of the aquarium they did so by regularly swimming about, resting longer each time they had progressed farther from the light and a shorter time between swimming intervals that again took them toward the light. A few came to rest in the lighter end of the aquarium, as almost always happens in light experiments.

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[17] *Amer. Jour. Physiol.*, 1903, Vol. IX., p. 282.

[18] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., p. 454.

[19] *Am. Nat.*, 1907, Vol. XLI., p. 684.

Now if the medusæ do not swim directly toward weaker light and are not turned definitely in such direction, even after trials, at any time, their collecting in weaker light might still be accounted for by the above explanation, if this could be elevated to the dignity of a method. That is, each time a medusa gets into an optimum light it remains longer, and when in an unfavorable light field remains a shorter time, and thus more and more of them will get together in these optimum places.[21]

From the foregoing it will be seen that there are several ways in which *Gonionemus* gets away from the strong light into an intensity best suited to its activities, without the intervention of tropism or "trial." If its only mode of locomotion, or even the chief one, to stimuli were the up-swimming "surface reaction" then it would plainly be "trial," or "motor reaction."

Change of Intensity as a Stimulus.

In Yerkes's earlier statements[22] about the relations of *Gonionemus* to light the words increase and decrease of light intensity are used, but only in his later answer to Morse's criticisms[23] does he make the statement that he has "abundant evidence that change in intensity of light stimulates the medusa." I had experimentally come to the same conclusion.

When the medusæ are at rest darkening the aquarium or shading one with an opaque object, such as the hand, is sufficient to start movement in a few seconds; again, by throwing stronger light with a small mirror on any medusa it can be made to move about. Indeed, the one or the other of these ways was generally used in my experiments to get motor reactions. Next some experiments were made to determine the height to which *Gonionemus* would swim after a single stimulation by change of light intensity. Eight medusæ were placed in a hydrometer jar 72 cm. deep and 9 cm. in diameter. When they had come to rest in the bottom of the jar they were stimulated by darkening the room until the medusæ could just be seen. They swam to the top, at intervals, somewhat irregularly. The shades were now quickly raised and thus the top of the jar illuminated with strong diffused daylight. Three medusæ swam downward so directly as not to touch the sides of the aquarium — not varying 9 cm. from their course. Five floated down part way and then two turned and swam the last third of the way down. In another trial, out of three at the top one swam down 31 cm. without touching the sides of the glass jar. This, it would seem to me, is in the nature of proof that change of light intensity starts and gravity directs the downward course.

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[20] Once I observed a medusa that seemed to follow pretty regularly a movable slit admitting stronger light into a darkened aquarium. Although it was pronounced I could not confirm the reaction in other specimens.

[21] After writing the above I find Mast ("Light Reactions in Lower Organisms, II., Volvox," *Jour. Comp. Neurol. Psychol.*, Vol. XVII., p. 169) has similarly explained the aggregation of *Volvox* in optimum light.

[22] *Amer. Jour. Physiol.*, 1902–3, Vols. VI. and IX.

[23] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., p. 460.

Some Mooted Points.

In regard to the question whether the decrease or the absence of light (darkness) is a stimulus for motion or inhibition there is difference of opinion. Yerkes in his first paper[24] on *Gonionemus* says "Romanes's statement that change from light to darkness is inhibitory of action is not very apt." He adds, it "is merely the absence of any motion producing stimulus." In a later paper on this medusa, however, he[25] says "decrease in light intensity temporarily . . . inhibits activity." Morse[26] from practically the same experiment that Yerkes used concludes that "we have no inhibition of movement in passing from light to darkness. In the dark the stimulating effect of light is absent and hence the movements ultimately cease." Yerkes[27] does not agree with this criticism but modifies his former statement to say "a considerable decrease causes a more gradual cessation of activity." I have no doubt that he has the correct solution of the question, as shown by the experiments above, when he says — "the change in intensity of light stimulates the medusa." In other words, then, a change in light intensity not only stimulates a resting medusa to move but it may bring a moving individual to rest. This is in accord with well-known facts in the behavior of other animals.

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[24] *Amer. Jour. Physiol.*, 1902, Vol. VI., p. 445.

[25] *Amer. Jour. Physiol*, 1903, Vol. IX., p. 282.

[26] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., p. 453.

[27] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., p. 460.

This leads to another point under discussion, *i. e.*, whether or not "the reactions of a swimming organism are different from those of one at rest."[28] Morse[29] believes they are not and supports his contention with an experiment of letting light fall on one half of a medusa resting in the dark; then on the half of a swimming individual. In swimming up both turned from the vertical. It is clear that the discrepancy is based on a misapprehension. To take Morse's own case as an illustration: the light let fall on the resting medusa set it in motion and beyond this, the light produced the same reaction, as it was really in each case falling on a swimming medusa. Therefore Yerkes's statement above is correct.[30]

Surface Reaction.

While there is no doubt that the up-swimming of *Gonionemus* is directed by gravity as stated by Yerkes,[31] nevertheless light seems to be a more important factor than he holds. Indeed I may say it is a necessary concomitant as may be seen from what follows. That it is not directive Yerkes (page 281) has shown by his experiment of using bottom illumination. The medusæ move up to the surface and turn over normally. But casual observation of the upper surface of the water shows that it is sufficiently illuminated from the bottom to allow the medusæ to come to the top. So I substituted lateral illumination through an opening near the bottom of the aquarium. The aquarium was otherwise darkened on all sides and reflection reduced as much as possible by filtering the water. Now the medusæ swam near the bottom, 8 cm. being the greatest height reached. As a control, bottom illumination was then used and immediately one of the animals swam to the surface and one came near it. Three other experiments were made, and the most striking case was that of one medusa swimming through the light band fourteen times in 72 seconds without reaching top or bottom. When the aquarium was so much darkened that I could not see the medusæ, sudden illumination showed that they had attached by their tentacles to the sides or bottom. Thus the presence of light seems necessary for the regular up-swimming activity of *Gonionemus* and gravity then acts as a stimulus to direct it.

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[28] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., Morse, p. 452; Yerkes, p. 462.

[29] *Amer. Nat.*, 1907, Vol. XLI., p. 683.

[30] Other points are covered in foot-notes 3, 10, 15, pages 356, 357, 359, also page 365.

[31] *Amer. Jour. Physiol.*, 1903, Vol. IX., p. 281.

In regard to the part light plays in the surface reaction, Yerkes[32] says: ". . . although light seems to be one of the important conditions for this reaction, it may occur in the absence of light." My experiments just cited show that the last part of this statement is not tenable and that the first part is correct. The chief importance of light in bringing *Gonionemus* to the surface, it seems, is in keeping the medusa negative to gravity.

Morse[33] incorrectly, believing that Yerkes held that light causes the inversion at the surface, denies this, and concludes "the cause for reaction is not evident." Later[34] he explains the inversion the same as Yerkes had previously done[35] by assuming that the apex of the bell is thrust unevenly above the surface. Now as the apex of the bell is heavier we no doubt agree that gravity causes the inversion, though not as a stimulus.

As the inversion at the surface is preceded by inhibition of contraction the question arises whether this is due to strong light. Yerkes[36] and Morse[37] have observed that medusæ do not stop and turn (invert) when made to swim up against a heavier substance than air, such as a board, *a glass plate*, a layer of olive oil, but they continue to swim against these layers until exhausted. The fact that inhibition and turning do not take place under glass indicates that light does not cause either. The above observations suggested to me that contact of the apex of the bell with air may cause cessation of movement. This idea is apparently supported by holding a layer of air imprisoned under a petrie dish cover some distance below the surface of the water. The animals respond the same as at the surface. Nevertheless, longer observation of the behavior suggests that it is after all, perhaps, nothing more than the recoil of a last ineffective stroke; something as when a person, finding one less step than he expected at the top of a stairway, does not immediately contract his muscles for another step but loses his equilibrium. Light not being the cause of the inversion (with Morse[38]), nor of inhibition, no further discussion is warranted here.

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[32] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., p. 458.

[33] *Jour. of Comp. Neurol. Psychol.*, 1906, Vol. XVI., p. 451.

[34] *Amer. Nat.*, 1907, Vol. XLI., p. 686.

[35] *Amer. Jour. Physiol.*, 1903, Vol. IX., p. 281.

[36] *Amer. Jour. Physiol.*, 1903, Vol. IX., p. 281.

[37] *Jour. Comp. Neurol. Psychol.*, 1906, Vol. XVI., pp. 450, 451.

ARE THE MEDUSÆ DIRECTED BY LIGHT RAYS?

Morse[39] has decided from an experiment with oblique illumination over the end of a shaded aquarium, because the medusæ collect in the ray-direction-end of the aquarium rather than in the shaded end, that "the direction of the ray of light is the important factor in orientation." Has he not left out of consideration another important factor, that of light intensity in the aquarium? Change of intensity has already been shown to be the important stimulus in reactions to light, nevertheless, it seemed worth while to test whether it is ray direction or intensity that determines where the

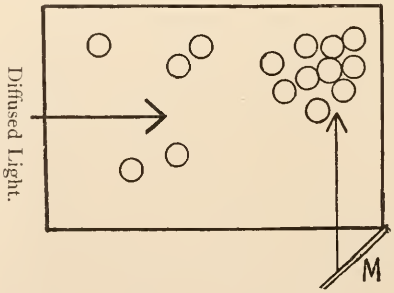

FIG. 2. Top View.

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[38] *Jour. Comp. Neurol. Psychol.*, 1906, Vol. XVI., pp. 450, 451.

[39] *Amer. Nat.*, 1907, Vol. XLI., p. 684.

medusæ will collect. The following experiments were tried: The aquarium used in previous experiments was placed before an open west window at three o'clock in the afternoon, in good diffused daylight. In fifteen minutes the majority of the medusæ had retreated to the end of the aquarium away from the window (Fig. 1, p. 356). Now a large mirror (*M*, Fig. 2) was placed vertically against one corner of the aquarium away from the window, so as to throw light across the end of the aquarium. In fifteen minutes most of the medusæ had collected in the opposite corner of the aquarium away from the window and where the light was least intense. The mirror was now placed at this corner and record again made in fifteen minutes. Many of the medusæ were scattered about, but again there was a larger number collected in the corner farthest from the sources of light. In two other experiments a sort of spot-light effect was produced, by having the aquarium darkened except at one end, and a vertical strip (Fig. 3) at one side next the open end so as to throw sunlight across the open end diagonally. There was also a slit near the top of the closed end of the aquarium for a band of sunlight or a mirror beam. The medusæ gathered farthest from the source of light when sunlight was passed through the end of the aquarium. Now crossing this sunlight by a beam from a small mirror, through the slit, made the medusæ leave the place where the light overlapped — where it was more intense. The position of the sunbeam and mirror was reversed with a corresponding result. In this case the mirror was held high enough so that its circumscribed area of reflected light fell on the farther corner of

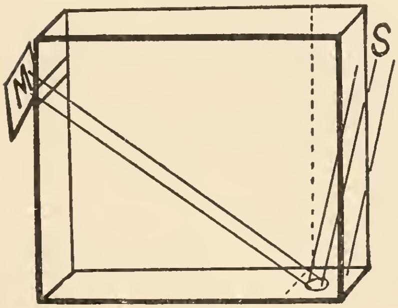

FIG. 3.

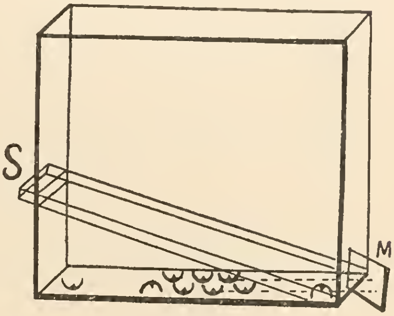

FIG. 4.

the aquarium, leaving a place of high intensity where the band of sunlight fell, but not so high as that where the two overlapped (Fig. 3). Other portions of the aquarium seemed to be of too low intensity, as the medusæ remained in the lighted portions. The effect of relative intensities was then seen by lowering the mirror so that its beam was projected horizontally across the band of sunlight. Again there were more medusæ in the corner farthest from the two sources of light (compare Fig. 2). In the final test, sunlight was admitted through a slit near the bottom of the aquarium and the mirror placed back of the aquarium in such a position as to throw reflected light obliquely against the sunlight, as it were (Fig. 4). Now the medusæ collected some distance from the back of the aquarium in the region of lesser intensity. Finally, that it is relative intensity and not ray direction is also shown by a former experiment (page 359) where medusæ turned away from an oblique band of sunlight in a darkened aquarium. They turn nearly as often toward as from the source of light. Ray-directing, seems to me, out of the question.

Summary.

1. The medusæ do not usually direct their movements to favorable locations but continue swimming at random until they come into an optimum environment, where they settle down.

2. Intense light turns medusæ away, thus avoiding injury.

3. Change of light intensity is the stimulus for reactions to light. In pronounced decrease, the change of intensity causes inhibition.

4. Relative intensity in the field, not ray direction, determines the place of rest.

5. Light is necessary for the up-swimming activity, though not directive — this being due to gravity.

6. Contact of the bell with air and the accompanying recoil probably causes the inhibition that precedes inversion of the bell at the surface.

Marine Biological Laboratory,
Woods Hole, Mass.,
August, 1909.

Vol. XVII *November, 1909.* *No. 6*

BIOLOGICAL BULLETIN

NEW AND LITTLE KNOWN HYDROIDS OF WOODS HOLE.[1]

CHAS W. HARGITT.

During the summer of 1907, while engaged upon certain problems associated with the work of the biological survey carried on at the Fisheries Laboratory, I described several hydroids, some new, others more or less rare, in a paper published in the Biological Bulletin, January, 1908. During the following summer I was fortunate in finding a few others which, like the former, were in part new and in part hitherto unknown within the locality, and in one case at least, wholly new to American fauna. In the following account will be found such descriptions as seem called for in order to bring them definitely to knowledge as integral factors of the hydrozoan fauna of the region concerned.

Cladocoryne floccosa var. sargassensis.

In a mass of *Sargassum* which was picked up during the summer of 1907 in Vineyard Sound, bearing a rich hydroid fauna, I found a very minute hydroid which at first greatly puzzled me. It was intricately associated with other species, particularly with *Aglaophenia minuta*, and at first seemed to be a sort of nematophoric accessory of this hydroid, the small round heads of young specimens bristling with nematocysts having but little resemblance to an independent hydroid. But a more extended examination brought to light other and larger specimens, and soon it was found that the thing under examination was beyond doubt a very minute and apparently unknown species of hydroid. A series of developmental stages were found giving all conditions, from minute buds just arising from the stoloniferous base to others with mere buds of tentacles, with still others having growing tentacles from the base of a definite hydranth on to the fully developed hydroid with full complement of tentacles, etc. Fig. 1 shows the hydroid enlarged ten diameters, while Fig. 2 shows the hydranth greatly enlarged to show the peculiar branching and knobbed tentacles. With this much clear it was not difficult to trace its generic affinities under *Cladocoryne*, Rotch.[1] But it was doubtful as to its specific relations. Rotch had described a species, *C. floccosa*, found at Herm, near Guernsey, having a habitat on stones, and being 6–12 mm. in height. Perrier has also described a species, *C. simplex*, found on *Sargassum*,[2] but I have not had access to Perrier's book, and so am unable to form any definite notion of that species.

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[1] Contributions from the Zoölogical Laboratory, Syracuse University.

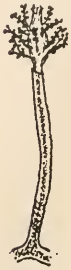

FIG. 1.

The present species is very minute, being only 2–4 mm. in height and differing more or less as to other features. I have suggested for it a varietal distinction, proposing the name *sargassensis*, as indicative of its habitat. The following characters are diagnostic:

Trophosome. — Stems mostly simple, occasionally branching slightly, rising from a reticulate hydrorhiza. Hydranths relatively large, spindle-shaped, with elongated hypostome similar to that of *Pennaria*. Tentacles about twelve, variously branched and definitely knobbed, and disposed in some three verticels over the body of the hydranth. These tentacles are peculiar and thoroughly distinctive, both in structure and development. A second series of oral tenacles, about six or seven in number, are simple, with knobbed ends, and surround the mouth. All are richly packed with nematocysts.

The perisarc, both of stem and hydrorhiza, is rather dense and irregularly annulated.

Gonosome. — This is wholly unknown, in the present specimens at any rate.

Habitat and Distribution. — The present is the only time I have seen the species. As stated before it has its habitat on floating *Sargassum*. I have hunted carefully over later collections of gulf weed but without finding trace of it.

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[1] *Ann. Mag. Nat. Hist.*, March, 1871, Vol. VII., p. 227; Allman, "Gym. Hydroids," p. 38.

[2] Cf. Billard, "Exp. Talisman," p. 161.

Calyptospadix cerulea Clarke.

On August 7, 1908, I found growing on the sides of the steamer Fish Hawk, at Woods Hole, several fine colonies of this hydroid, originally described by Clarke,[1] and so far as I am aware has not since been a subject of record. In general aspects and size it resembles *Bougainvillia*, and was in the present instance thought to be that hydroid. A closer scrutiny soon revealed its marked differences.

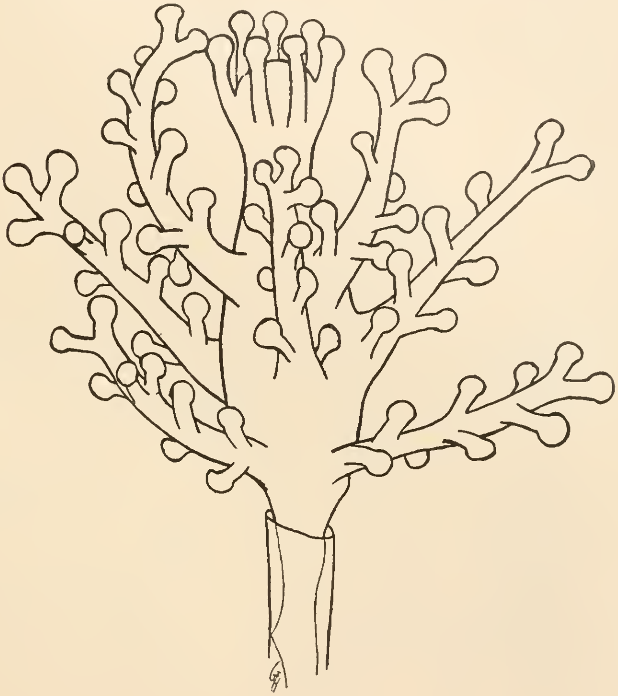

Fig. 2.

Its original description from Chesapeake Bay, and its occurrence on the Fish Hawk, which had only a month previous come from Norfolk, at once suggested the probability of its having been thus transported to this locality. It is not strange, there-

[1] *Mem. Bost. Soc. Nat. Hist.*, Vol. III., 1882, p. 136.

fore, that I made the following entry in my notes at the date above mentioned: "This is a fine illustration of the importance of ships as a means in the distribution of organisms." On the following day it occurred to me to look about the docks at which the steamer was moored as to whether any signs of the hydroid might be found on the piles; and somewhat to my surprise colonies were found at several points, some of them quite remote from the ship. Immediately the query arose, Did the Fish Hawk bring the hydroid, or had it found a place on the ship from contiguous piles of the dock? The smaller and younger conditions of colonies on the ship suggested the latter alternative, but still with the prepossession of theory strongly inclining to the former. An examination of the outer side of the ship showed an almost entire absence of the hydroid, which still further emphasized the doubt as to the ship's relation to the matter of distribution. The matter found a final solution so far as the *present* issue was concerned when on August 10th Mr. Vinal Edwards having at my request brought a few hydroids from Wareham bridge at the upper arm of Buzzards Bay, and I found among the material fine colonies of the same hydroid. This of course ruled out the Fish Hawk so far as the present case was concerned, for the last habitat was entirely beyond the reach of the ship as a means of transportation.

During the current season, 1909, I looked several times at the fisheries docks for colonies during July and early August, but in vain; but again I was able to obtain luxurious colonies from the Wareham locality. This clearly established the fact that the species is thoroughly established as a permanent feature of the local fauna. But the matter as to *how* and *when* it became established must be a subject of much uncertainty for the present. That it has been established for any considerable time I seriously doubt, having been collecting throughout the region more or less assiduously for many years without previously finding any trace of its presence.

The hydroid is a large and beautiful species, the bluish color of the female gonophores making it strikingly different from almost all other species of its character. Fig. 3, copied from Clarke's paper, gives a fair idea of the main features of the hydroid.

Clytia volubilis. Fig. 4.

On floating masses of *Sargassum* were found prolific colonies of a hydroid which had many of the characteristics of *Clytia johnstoni*, and which I took for a time to be that species, though recognizing certain features which differed from it. During the current summer I took at Harpswell, Maine, what proved to be very typical specimens of the species, and which upon comparison with the former showed very marked and constant differences. I was therefore forced to reconsider its specific relations. In doing this I had occasion to compare it with specimens taken at Naples several years ago, and which I had then considered as *C. johnstoni*. The two species had much in common, indeed differed hardly more than might species from remote localities. A review of the literature brought to light the fact that certain authorities have considered the two above named as identical. For example, in his monograph "Die Hydroiden des k. k. naturhistorischen Hofmuseums," Marktanner-Turneretcher has thus treated them, giving preference to the earlier name of Ellis and Solander.

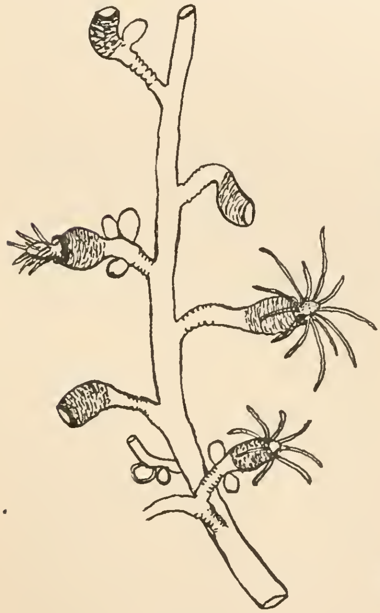

Fig. 3.

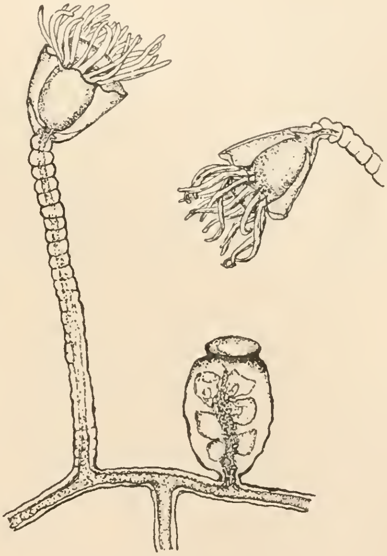

Fig. 4.

A comparison of the characteristic specimens of *C. johnstoni* taken at Harpswell with the Woods Hole and Naples specimens has led me to consider both as entitled to specific distinctness, and I am therefore designating the local species as *C. volubilis*, and believe the Naples specimens to be the same. The following features are diagnostic :

Stems usually simple and unbranched, 2–4 mm. high, annulated at proximal and distal ends, occasionally indefinitely annulated throughout. Hydranths relatively large, with 20–24 stout tentacles, and with a prominent hypostome, more or less trumpet-shaped in expansion. Hydrothecæ broadly campanulate, not very deep, and with about 10–12 shallow rounded teeth, in some specimens the margins hardly more than undulate.

Gonangia borne on the reticulated hydrorhiza, rather large, and with very short plain pedicels. An interesting feature was the fact of a remarkable variation as to the aspects of these organs. Most were rather smooth, oval structures ; but in not a few cases they were strongly corrugated throughout, and examples showing all phases of intergrading in this particular were easily found.

It may be well in this connection to call attention to a species of *Clytia* described by Congdon from Bermuda,[1] *C. simplex*, which has features in some measure intergrading with the one under review. I have not seen Congdon's type specimens, hence have only his general description as a guide. It will be seen that his specimens average considerably larger than my own, and the hydrotheca is given as longer, and with deeper teeth, still it might be worth an attempt to critically compare the types of these several species with a view to ascertaining just what grounds might be found bearing upon their interrelations.

CLYTIA CYLINDRICA Ag.

On at least two occasions recently I have taken this beautiful little hydroid. While at times it may be found in considerable numbers, it does not seem to be especially common, though this may be due in part to its very small size. In height the simple stems are from 1–1.5 mm. ; the hydrothecae about 0.5 mm.

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[1] *Proc. Am. Acad. Arts and Sci.*, Vol. XLII., p. 471, 1907.

long by about 0.2 mm. broad; they are cylindrical in form, with about 8–10 sharp, deeply cut teeth. Gonangia are elongate, more or less cylindical, smooth, borne on delicate pedicels ringed at proximal and distal ends. The hydranths are extremely delicate, and with delicate orange to reddish tints just below the tentacles.

OPERCULARELLA PUMILLA Clark.

Among a few hydroids collected in March, 1908, by Dr. F. B. Sumner were found a very few specimens of this species, a record of which is important since I can find no evidence of its occurrence since that of its original description by Clark.[1] He records having taken it at Portland, Maine, and off Montauk Pt., Long Island. The related species, *O. lacerata* Hincks, he records from New Haven, Conn. Clark expressed some doubt as to whether his species really came under the genus to which it was assigned, and Nutting has expressed doubt as to the validity of the species, believing it probably identical with *O. lacerata.* My own specimens conform very closely with Clark's description and figures. It is a most beautiful and delicate little hydroid. Stems and branches are annulated throughout. No gonangia were present on my specimens.

OBELIA CONGDONI, n. sp.

On several occasions recently I have taken from floating gulf weed at Woods Hole an *Obelia* which, while apparently closely related to *O. hyalina* Clarke, differs in several important features, as will be pointed out later.

Congdon has recently described a species from Bermuda, which he referred to Clarke's *O. hyalina*,[2] but which I am convinced is identical with the species under consideration, and which seems to me to be an undescribed species.

Congdon's description and figures are sufficiently accurate to obviate necessity for any considerable details in this connection (cf. *op. cit.*). A few points which seem to be in rather sharp contrast with Clarke's species may be given.

According to Clarke[3] the "branches of the stem arise in the axils of the hydrothecæ." This I do not find to be the case in the present species. Again, according to Clarke, the "gonangia are small, about twice the length of the hydrothecæ, rounded off at the distal end, with a simple spherical, terminal opening which stretches across the distal end." On the contrary, the gonangia are large, about four times the length of the hydrothecæ, and the opening is not simple, but there is a terminal neck with everted rim. It should also be said that in contrast from Clarke's species in which the colony is said to be "about 12 mm. in height, and but little branched," in the present case the colony is from 20 to 30 mm. in height, and much branched.

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[1] *Trans. Conn. Acad. Sci*, Vol. III., pp. 61–2.

[2] *Proc. Am. Acad. Arts and Sci.*, Jan., 1907.

[3] *Bull. Mus. Comp. Zoöl.*, Vol. V., 1879, p. 241.

Gonosome. — The medusæ when liberated have 24 tentacles, but others are rapidly acquired and within ten or twelve hours many specimens have from 30 to 36. The general aspects of the medusa are distinctively obelian ; there is the eversible bell, the squarish manubrium at base, with rounded oral portion, with two otocysts in each quadrant.

Regarding the species as new, and in deference to Congdon's description, I suggest as its specific designation *Obelia congdoni.*

Calycella syringa.

This species is neither new nor rare in this region. Reference is made to it for the purpose of calling attention to certain features of habitat and variation which seem of some interest and importance. Nutting refers to it as "found abundantly in the Woods Hole region, growing over all sorts of plant-like marine organisms, especially other hydroids." This statement I am able to confirm, though with a single qualification, namely, its seasonal oscillations. I have found it rather *uncommon* during the midsummer season, and have never found it actively propagating at this time by sexual modes. In early spring — March to May — it seems much more abundant and immense colonies with prolific crops of gonangia are not rare.

Another feature calls for some attention, namely, the variable size and aspects of the species in midsummer. At this time specimens found by me have been invariably of dwarfed character, so much so that for some time I was rather inclined to consider it as a distinct species. Typical specimens taken in spring have the distinctive elongated and spirally annulated pedicels and large hydrothecæ. But specimens taken in summer, so far as my observations have gone, are uniformly and constantly small — only about one fourth that of typical specimens, and have extremely short pedicels, with only one or two annulæ, or with none. I was not unaware that Clarke[1] had referred to certain variations in size, but he made no special reference to it save as an exception. It was only after careful search among colonies of typical specimens that I was able finally to find an *occasional* specimen of this dwarfed character. I have satisfied myself that it is but another instance of that tendency to seasonal variation which is well known in other cases. It is well, however, that it be emphasized, as well as the further fact that at certain times *dwarf features* are *distinctive* and *constant*. Fig. 5 shows some of these dwarfs enlarged.

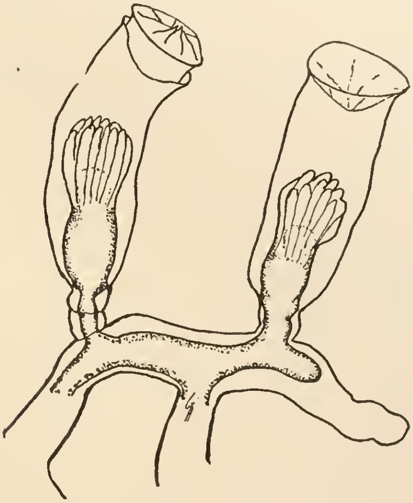

FIG. 5. × 100.

One other feature may be referred to in connection with this phase. Clarke called particular attention to the appearance in certain hydrothecæ of this species of a "wide ring, oramented with from ten to fourteen longitudinal markings, which rises for some distance above the rim and on the summit of which there is borne either an operculum or another ring; in some cases there are as many as four of these rings with an operculum at the summit." Such series of rings I have found to be rather common; but it has not been possible to distinguish, even with high powers, the "ornamental markings" to which Clarke makes reference. The surface of these secondary, or additional rings is quite as devoid of such markings as is that of the original hydrotheca itself.

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[1] *Trans. Conn. Acad. Sci.*, Vol. III., p. 66.

CALYCELLA NUTTINGI, n. sp. (Figs. 6, 7.)

Growing upon colonies of the bryozoön, *Bugula turrita*, taken at the fishing grounds off Sankety, and later at Woods Hole, and even still later at Harpswell, Maine, I have found a microscopic species of *Calycella*, which seems to be undescribed. It is hardly more than one tenth the size of an average specimen of *C. syringa*, and differs in other respects as well. Its very minute size may probably account for the fact of its having been overlooked in spite of continuous collecting throughout the region for many years. The following characters are diagnostic of the species:

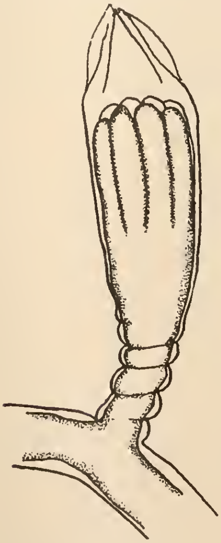
FIG. 6.

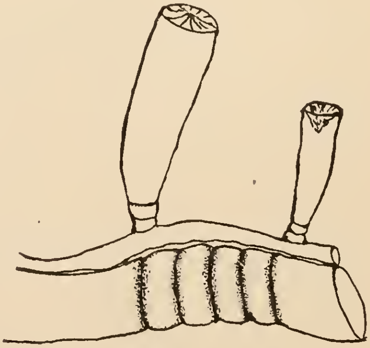
FIG. 7.

Trophosome. — Colony composed of a creeping, filiform stolon, slightly, if at all, reticulated, from which at very irregular intervals arise the hydrothecæ. These are tubular, though not quite cylindrical, gradually widening from base to margin, as shown in Fig. 7, and are without appreciable constriction at base where it articulates with the short, annulated pedicels, the annulations occasionally extending some distance (rarely over entire body), on the thecal walls, giving the impression of complete annulation when viewed obliquely. The hydrothecæ are very delicate, often collapsing at the distal ends when being prepared for mounting. There is a definite operculum, which often appears plaited, the individual valves being more or less difficult to distinguish. I have not determined their exact number with any degree of certainty. In many cases these valves exhibit the same aspect of inversion as is the case with *C. syringa*, but I have not found the presence of secondary rings or other marginal duplication as in the latter species. Total length of pedicel and theca 0.2–0.3 mm. or an average of about 0.25 mm., by about 0.07 mm. in diameter.

Hydranth extremely small and delicate; body elongate, cylindrical, with conical hypostome; tentacles very delicate and thread-like, usually ten in number, occasionally eight.

Gonosome unknown. The examination of many colonies from various localities failed to discover signs of gonangia. It may be probable that like *C. syringa* this species has its breeding season at some other time of year.

Habitat. — Found only associated with other hydroids, or similar organisms, *e. g.*, bryozoa, and hence is probably of commensal habit. No evidence was found indicating parasitism.

It is a pleasure to name the species, with his consent, in honor of my friend and distinguished student of hydroids, Professor C. C. Nutting.

KERATOSUM COMPLEXUM, n. gen. and sp. (Figs. 8–10.)

On three successive summers there has been taken an organism at Crab-ledge which was variously assigned to the Porifera, Bryozoa, and finally came to the writer. A glance at Fig. 8 will show how little there is from a superficial view to suggest hydroidean affinities. Indeed it was only after sections had been made, or maceration and dissection of the thing, that its true relations became evident. And it was only after considerable research that its generic relations were even approximated. In 1892 Levinsen described a hydroid from Greenland (Meduser, Ctenophorer og Hydroider fra Grönlands Vestkyst),[1] which seemed to have much in common with the one here under review. He had described it as a new species under the genus *Lafœina*, Sars, naming it *L. maxima*. At first it was thought the present species was probably identical with it, but when one undertook to work out details of morphology it became more or less certain that it not only was not the same species, but that, moreover, it could hardly belong to the same genus, if, indeed, there might not be the necessity of establishing for it a new family.

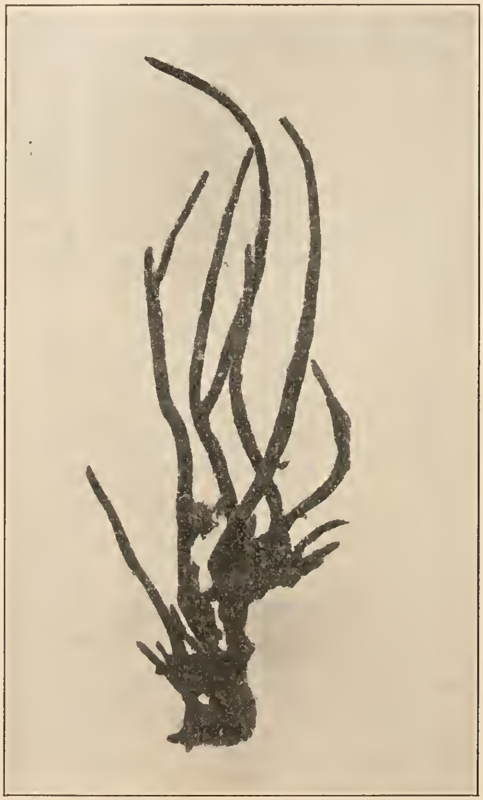

FIG. 8. Photograph of colony ⅔ natural size.

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[1] "Saertryk af Vidensk. Meddel. fra den naturh. Foren.," 1892.

The genus *Lafœina* was established by Sars (Bidrag til Kundskaben om Norges Hydroider)[1] for a very minute hydroid found on stems of *Perigonimus*, the chief generic character of which was the presence of minute urticating organs, or nematophores, unlike any before known. Levinsen's description is rather inadequate, and his figures not altogether satisfactory, but to the writer there seems to be so comparatively little in common between his species and that of Sars, that it may be doubtful whether it should not have been placed under a new genus. Be that as it may, it seems very sure that the present one must find different generic housing. For example, in Sars' genus the hydroid has a reticulate hydrorhiza, and Levinsen describes something of the sort for *L. maxima*, but in the present species while there may be comprised something of the sort, it would be more correct to describe the complex stem as arising from a dense, sponge-like base, etc.

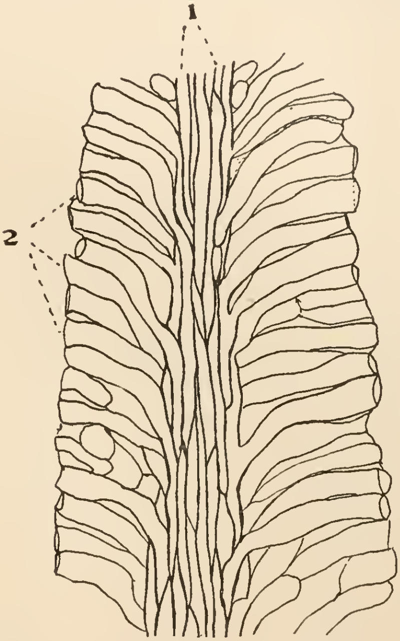

FIG. 9. 1, Axial tubes; 2, hydrothecæ.

Concerning the family relations I am not disposed in this connection to enter into any critical review. While the Perisiphonidæ would be the only one under which it might be placed, still the family as at present defined, according to Allman (Hydroida, Part II., p. 32),[1] would by no means provide for the species. For example, while there is an axial tubular mass, as shown in Fig. 9, there is no single one of these which bears the hydrothecæ as called for by the definition referred to. However, for the time being the species may be left under this family till such time as adequate revision may be undertaken, when the needed modifications may be provided.

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[1] "Saerskilt aftrykt Selsk. Forhandlinger" for 1873.

As already intimated, it seems necessary to institute a new genus as well as species for our hydroid. For the genus characters the following are designated as diagnostic :

Colony sponge-like, both in general aspect and in the texture of stems and branches, as well as in growth-habit. Looked at from a short distance it resembles very much our common sponge, *Chalina arbuscula*, in almost every particular. Hence the proposed generic name — *Keratosum*. The stems arise from a disk-like spongy base and branch much after the manner of "finger sponges." These are composed of a complex and intricate mass of siphon-like tubes which ramify and anastomose irregularly, and from which arise hydrothecæ, and nematophoric organs, the latter with thecoid terminal structures similar to the former, the whole cemented together by a dense sponge-like felt of very tenacious and resistant character. Longitudinal and transverse sections of stems or branches show them to be composed of the following parts : (1) a central, axial portion, made up of more or less parallel, anastomosing tubes ; (2) a peripheral portion, composed chiefly of hydrothecæ and what may be termed nematothecæ ; (3) ramifying strands of cœnosarc, which seem to interpenetrate the elements of the peripheral zone. Figs. 8, 9 and 10 will show both the surface aspects as well as sectional views just mentioned.

Concerning specific diagnosis it must be regretted that the physiological state of the hydroid was such as to afford but meager characters of specific nature. The organism in all the specimens collected seemed to be in a state of hibernation, or better, perhaps, *æstivation*, no hydranths or similar organs being distinguishable. Hence such organs as tentacles, gonophores, etc., which afford important specific characters, were wholly lacking. I had at first attributed this condition to bad preservation; but collections made at two subsequent seasons, in each case care being taken to preserve by approved methods, have convinced me to the contrary. It seems highly probable that this hydroid during the summer season is in a state of suspended animation, so to speak; a condition quite common among hydroids at various seasons. It must suffice in this connection to make brief reference to a few features, as hydrothecæ, etc. As shown in the figure, the hydrothecæ are tubular structures, arising from the axial tubes by rather narrow necks, and extending upward and outward, becoming more or less curved, and opening to the surface by somewhat oblique mouths. While in many cases there seemed to be opercular-like folds at the thecal openings yet they were difficult to definitely demonstrate or describe. As to size hydrothecæ averaged about 0.7 to 1 mm. in length, by about 0.12 mm. in diameter at median portion, somewhat larger at mouth. In no case were gonangia distinguishable, nor evidences of germ cells. This might be expected as to the last, but if gonangia are an organic part of the skeleton one might

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[1] "Report Chall. Exped.," Vol. XXIII. (part 70), p. 32, 1888

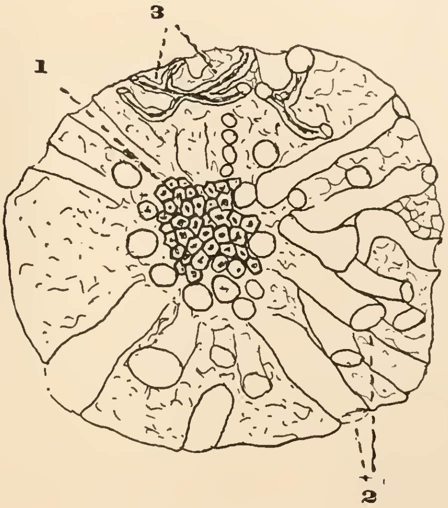

FIG. 10. Cross-section of stem. 1, Axial tubes; 2, hydrothecæ; 3, cœnosarcal strands.

expect some trace of them in some specimens, at any rate. But none could be recognized.

Nematophores were distinguishable, and in a general way seemed similar to those described by Levinsen. They are elongated structures, cylindrical in shape, and with terminal thecoid organs which are smaller than the hydrothecae, much smaller indeed, but with no peculiar or distinguishing features. In many cases the definite organization of the nematophore was distinguishable, and the knobbed heads were found loaded with numerous nematocysts which measured about 0.015 to 0.02 mm. in length by about one third of this in diameter. In shape the nematophores may be designated as elongate-clavate ; and are probably protrusible in life beyond the nematothecæ as organs of defense, or offense, according to circumstances.

In connection with the account of the morphology there should have been mentioned a matter of interest, as well as of difficulty, namely, that concerned with the attempts to dissect and separate the elements of the complex stem structure. The usual resort to boiling with potash or caustic soda, while affording some aid in clearing out the organic contents of the tubes, afforded very small aid in isolating the elements. Even when macerated for hours or days in strong solutions, or after prolonged boiling, so far as my own efforts were concerned, the macerating processes availed but little. And when resort was had to javelle water the consequences were worse, for with that agent both the cement substance and the chitinous perisarc itself were attacked about equally, and the end was, naturally, the disintegration of the entire mass.

I was interested to find a similar experience recorded by Allman (*op. cit.*, p. 47). Of the adhesion of the tubes of *Grammaria* he says : "So intimate is this adhesion that I have found no treatment, even prolonged boiling in caustic potash, of in any way overcoming it. *Grammaria* in this respect presents a striking contrast to *Cryptolaria*, as well as other genera of the Perisiphonidæ, in all of which maceration in a solution of caustic potash so weakens the adhesion of the tubes to one another that they may then be easily separated by the dissecting needle."

All in all, we have in this hydroid one of the most interesting, and in some ways anomalous, of this remarkable group of organisms. No name compatible with the rules of nomenclature would in any measure serve to more than hint somewhat of this; hence in proposing for it the above title — *Keratosum complexum* — it may be presumed to be modestly christened!

It is a pleasure to acknowledge obligations to Dr. R. C. Osburn for aid in securing material of this species.

Syracuse University,
September 15, 1909.

AN ECOLOGICAL STUDY OF THE PLANKTON OF SHAWNEE CAVE.

WITH NOTES ON THE CAVE ENVIRONMENT.[1]

WILL SCOTT.

WITH THREE FIGURES.

During the year beginning September 7, 1907, the speleological fellowship of the department of zoölogy of Indiana University was held by the writer with residence on the Cave Farm of the University, three miles east of Mitchell, Ind.

A preliminary examination of the cave stream revealed the presence of considerable plankton. A systematic collection of the plankton was immediately begun and after some time a quantitative method applicable to the cave was developed.

The organisms constituting the plankton were found to be epigean forms. It developed that the seasonal distribution of the cave plankton is different from that of epigean streams and lakes. Its maxima and minima seemed more closely related to stream level than to any other factor of the environment.

These facts led to an examination of the surface of the region overlying the cave, in order to determine if possible the source of the plankton, and the relation of its source to its distribution in the cave. It was found that the plankton is derived from ponds in sink-holes of a particular type, and that its source is a primary factor in determining its distribution.

After the plankton enters the cave it is modified in various ways by its new environment. The cave environment is divided into two distinct regions, the terrestrial and the aquatic. These two regions are alike in that there is an absence of light in both, and that each influences the temperature of the other. They are also both affected by the form of the cave.

Some of their differences aside from their primary ones are: (1) the temperature of the air approximates the temperature of the walls of the cave very soon after entering it, while the temperature of the water approaches this constant slowly, (2) the rate of the air current in this cave is determined by the outside temperature, while the rate of water current is determined by its level, which in turn is determined by rainfall. Since the terrestrial region affects the plankton-inhabited aquatic, both regions need to be considered.

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[1] Contribution from the Zoölogical Laboratory of Indiana University, No. 104.

A careful examination of the bed of the stream was made for sessile and bottom-inhabiting forms with negative results.[1]

Previous Work. — Previous studies on the microörganisms of caves have been confined for the most part to the enumeration and description of species. Claus, Schmeil and Joseph have investigated the zoöplankton of some of the European caves and have recorded the species.

In this country records of microörganisms inhabiting cave water have been made by Tellkampf ('45), Packard ('89), Banta ('07) and others. Kofoid ('99) has described a towing net collection taken in Echo River, Mammoth Cave, by Eigenmann. This catch contained twenty species. Ulrich ('02) reported twenty-one species from the water of an artesian well at San Marcos, Texas. Among these were two species of cyclops, both of which he regarded as new.

The Cave. — The cave[2] in which these studies were made is at present a rather simple tube with a few side passages. A stream of some size flows through its entire length. The openings of the cave are in Section Four (4), Township Three (3) North, Range One (1) East. These openings are five in number. The lower one is the outlet and is known as Shawnee Cave. The four other openings have been formed by the collapse of two sections of the roof and are known as, Lower Twin Cave, Upper Twin Cave, Lower Dalton Cave, and Upper Dalton Cave, respectively (see map).

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[1] I am greatly indebted to Dr. C. H. Eigenmann, professor of zoölogy, for his helpful criticisms and the loan of literature from his private library; also to Dr. Charles Zeleny, associate professor of zoölogy, for valuable suggestions. Messrs. F. C. Greene and N. E. McIndoo assisted in surveying the cave. They are entirely responsible for section II.

[2] The upper end of this cave is not known. It has been explored to a point about two miles above the outlet. At this point a mass of fallen rock nearly closes the passage. Mr. N. E. McIndoo has crawled through this passage, but it is too small to admit the boat. A stream, which drains a valley some two miles long, flows into an underground passage about four and one half miles southeast of the outlet of Shawnee Cave. This is known locally as "Mosquito Sinks" and is indicated by Newsom ('01) in section sixteen, township three, range one north. Possibly this is the upper limit of the cave. The openings where the stream "sinks" are too small to be explored. Five hundred wooden blocks, which had been soaked in paraffine, were placed in these openings, but none has been taken within the cave, to date.

Vertically the cave is located in the Mitchell limestone, and has been formed by solution along seams in the rock. These seams follow approximately cardinal directions and hence cross at right angles. The result of this is that much of the cave consists of straight passages at right angles to each other. The erosive action of the stream being much greater in the eddies at the turns than in the straight passages deep pools are formed at these points.

Obstructions. — The cave stream has been obstructed more or less completely at four points in the region that I have explored. The upper obstruction has been formed by the collapse of the roof, possibly below a sink-hole, between the Dalton caves (I–36, 37).[1] The second in a similar manner between the Twin caves (I–32, 33). A large part of the upper dam has been removed by solution and erosion, so that the stream flows over it.

At the Twin caves the obstruction is complete at ordinary stages of the stream. It occurs at a right-angled turn in the cave. A new passage is being formed cutting off the obstructed corner. The lower end of this new passage is a few feet below Lower Twin Cave, the location of its upper end is not yet positively determined. The passage is too small to be explored and insufficient to accommodate the stream in times of flood; in such times, the water flows over the obstruction between the two caves and resumes its original course.

The third obstruction is 1,400 feet below Lower Twin Cave at the so-called "Big Room" (I–15, 20). Here a number of old caves crossed the present stream at a higher level. The strata between these two cave levels fell and for a time completely dammed the stream. Deposits of gravel and clay were then formed above this point. In the side passages that are protected from erosion by the cave stream, the deposits still reach the roof. Much of the obstruction and resulting deposits have been removed by the cave stream.

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[1] Numbers refer to map.

Eight hundred feet below the "Big Room" is the fourth obstruction (I–13). The roof has fallen from some cause that I was unable to determine, and has completely dammed the stream. A new channel has been formed from this point to the outlet, except for a distance of 40 feet, where it flows through an old cave. The direct result of these obstructions is the formation of pools having a maximum depth of about 10 feet.

Elevation. — The outlet of Shawnee Cave is 40 feet above the level of White River which is about two and one half miles distant. The Lower Twin Cave is 30 feet above Shawnee Cave and Upper Dalton Cave is 10 feet above Lower Twin Cave. The gradient of the stream in the lower part of the cave is 40 feet to the mile. It falls very rapidly from the outlet for about 800 feet, and then has a gradient of about 12 feet to the mile. Probably this slight gradient and the close approach of the stream to local base level prevents the stream from finding a lower level when obstructed, thus causing the effect of the obstruction to continue.

As a result of the pools at the turns and above the obstructions, the water in the cave may be divided into a *constant* which is the amount of water in the pools at all times and a *variable* which is the amount of water flowing through the cave. The ratio between the constant and the variable is much greater in the lower (ordinary) stages of water than in times of flood. At ordinary stages of water, it requires a given particle of water much longer to pass through the cave than if the cave were a straight tube.

Five hundred wooden cubes which had been soaked in paraffine were put in at Lower Twin Cave, and a trap of "hardware" netting (one half inch mesh) was set at Shawnee Cave. Fourteen days later the first blocks were caught at Shawnee Cave. In times of flood the current in very rapid. Just what the rate is could not be determined as no trap could be designed that would withstand the terrific force of the stream at Shawnee Cave. Certainly not more than a few minutes are required for water to pass from Twin Cave to Shawnee Cave in times of flood.

It is evident, then, that the plankton of the cave is subjected to the cave environment for a much longer time during the lower stages of water than during the higher stages. This has a marked effect upon the number of species and the number of individuals constituting the plankton at the different stream levels.

Light. — This cave is like most others for the greater part of its length, in that there is an absence of light. It differs from most others, in that the stream is illuminated at the points where the roof has collapsed. The stream is fairly rapid in the sections exposed to light, so that the illumination is of short duration. The illumination is strong between Dalton caves and scarcely more than twilight at low stages of the stream between Twin caves. These short exposures to light may enable some of the zoöplanktonts to feed and cause some carbon assimilation in the phytoplanktonts. The effect is probably very slight.

Temperature. (*a*) *Air.* — The temperature of the air of the interior of the cave is between 52–56° F. throughout the year. The temperature has been recorded for the last two years on a thermograph stationed fourteen hundred feet below Lower Twin Cave. The variation exceeds the error of the instrument slightly. Where the air flows out at an opening it differs in observed cases less than one (1) degree Centigrade from the temperature of the interior. Inflowing air assumes the temperature of the air in the center of the cave gradually.

On September 7, 1908, a Centigrade thermometer carried into Lower Twin Cave showed that the interior temperature was reached 428 feet from the opening. During extreme temperatures above ground this point would be farther from the opening.

(*b*) *Water.* — The water temperature varies much more than that of the air for obvious reasons. During low water the temperature of the water approaches the temperature of the walls of the cave. During a flood, it varies toward the temperature of the water outside the cave. Floods, then, cause the temperature of the cave water to lower in winter, to rise in summer, but affect it slightly when the outer temperature is near 54° F. The variation is much less in summer than in winter, because the summer floods are not so great.

In every flood observed, the extreme water temperature occurred about twenty-four hours or more after the crest of the flood. This may be due to the influence of the residual water in the cave (see Table I.).

TABLE I.

SHOWING THE RELATION OF FLOOD TO TEMPERATURE OF CAVE WATER.

Winter.

Date.	Water Temp.	Outer Temp.	Crest of Flood.	Rainfall.
Dec. 12	11.6°+C.	< 0°C.	Crest	1.48
" 13	11.5°+C.			
" 14	8.7°+C.			
" 15	8.0°+C.			
" 16	9.4°+C.			
" 21	rises to 11.0°+C.			
		Summer.		
Aug. 13	12.5° C.	max. 32.7° C. min. 21.1° C.	Crest	2.20
" 14	12.5° C.			
" 15	12.5° C.			
" 16	12.6° C.			
" 17	12.6° C.			

Air Currents. — The air in the cave is in almost constant motion. In general it flows up during the winter and down during the summer months. However, during the spring and autumn, the current reverses several times before the constant direction of the extreme season following is assumed. The direction of the current was down on October 14, and reversed on October 21, 22, 23, 26 and 30. After December 10 the air moved upward until the unstable period of spring began, which occurred about the end of March.

The rate of the current varies in different parts of the cave, being scarcely perceptible in the large rooms and very marked where the cave is small in cross-section. Observations taken at a single point indicate that the rate of the air current varies directly with the divergence of the subterranean and surface temperatures. Rate of air currents was measured with a meter recording a minimum of 30 feet per minute.

The upward moving current of cold air weathers the rocks in the regions of the lower openings of the cave, giving them in general a funnel shape. In this enlarged region there is always an upper stratum of air moving in the direction opposite to that of the primary cave air current.

The closed passages near Shawnee entrance have the ordinary convection of a closed room, *i. e.*, a lower and an upper current moving in opposite directions. When the air currents were too weak to be measured, their direction was observed by means of a candle flame.

These results confirm and elaborate the results of Banta ('07) and Egli ('04). Banta found by weekly observations in Mayfield's Cave that the air currents reversed about October 1 and April 1. Egli found in Höll-Loch, a cavern explored by him, that the currents were down when the outer temperature was 8.1° Centigrade, and up when 2.3° Centigrade or below, being very strong at — 8° Centigrade.

Method. — It is much more difficult to collect plankton in a cave than in ordinary waters. The lack of light, the dimensions of pools, the relatively small amount of plankton, the great and sudden variations in the cave stream, and the necessity of transporting and manipulating the apparatus unassisted, rendered many of the ordinary methods impracticable.

After various experiments the method finally adopted for all quantitative work was as follows: A net was constructed of bolting silk No. 20 (Dufour) after the pattern described by Kofoid ('97). The net was suspended in a pool with about 3 inches of its filtering surface exposed. Two hundred gallons of water were dipped and poured into it with a bucket (1 ½ gal. capacity) at the rate of 10 gals. per minute. The catch was preserved in a 4 per cent. solution of formalin. I am well aware that this method results in some error (Kofoid, '97), but it was the most practicable for this investigation.

For the examination of material, glass troughs were constructed by cementing glass strips on a slide, with marine glue. The width approximated the diameter of the field of the microscope (⅔ obj., 2 in. ocu.), the length 40 mm., and the depth about 1 mm. The material was pipetted into this trough until the surface film was parallel to the slide. The entire catch was counted in quantitative work.

When higher powers of the microscope were necessary for careful study, the material was removed from this trough to a slide on which strips of cover glass were glued for the support of the cover glass. After examination, the material was washed from the trough into a new vial with 4 per cent. formalin.

Amount of the Plankton. — The amount of plankton is small when compared with the amount taken in a lake or epigean stream. In low stages, 200 gallons contained less than 1 c.c. In times of flood the catch was increased very much (200 c.c. or 300 c.c.), but this increase was largely due to silt and vegetable débris carried in suspension.

Constituents of the Plankton. — No organisms were found that were not referable to known epigean forms. Rather marked variations from the type were observed in some of the crustacea, notably in the genus *Bosmina.* However, the recent work of Wesenberg-Lund makes it advisable to withhold judgment on this point, until the local and seasonal variations in the crustacea of the ponds of the region have been investigated.

The zoöplanktonts taken included Copepoda, Cladocera, Rotifera and Protozoa.

The phytoplanktonts belonged to the following genera: *Spirogyra*, *Zygnema*, *Volvox*, *Closterium*, *Micriasteria*, *Vaucheria* and *Pandorina.* In addition to these, diatoms, worms, insect larvæ and fragments of spiders occurred rarely.

The planktonts having the widest temporal distribution were *Bosmina cornuta*, *Cyclops bicuspidatus* and *Anura cochlearis.* *Cyclops prasinus* outnumbered *Cyclops bicuspidatus* in November and several species of Rotifera were quite common in May.

The loricum probably enables rotifers to withstand cave conditions as all the Rotifera except two belong to the suborder Loricata. These two were found when the current was quite rapid and disappeared when the water became low and the current reduced. There may be other factors in the organization of the Loricata besides the loricum that enables them to exist under these conditions. That the rotifer fauna of the cave is included with rare exceptions in this subclass is certainly significant.

Diatoms were very rare. This was probably due to two things — (1) these forms live on the bottom of the ponds and consequently few are carried into the cave, (2) they soon succumb in the cave.

Other algæ were never taken in large quantity, but *Volvox* and *Closterium* were present in most of the catches throughout the year. Filamentous algæ were found at Shawnee Cave only in the higher stages of the stream.

The Source of the Cave Plankton. — To understand the distribution of the cave plankton, it is necessary to understand its source. It has been assumed that the plankton (at least in part) inhabiting a cave stream has been derived from the surface through sink-holes.[1] An examination of the local sink-holes made it clear that probably only a small part of the total number contribute.

Sink-holes may be divided into two principal classes, compound sink-holes and simple sink-holes. A compound sink-hole is a large depression having secondary sinks on its slopes. These arise in most cases probably by the reduction of the divides of the compound sinks by erosion. Sinks may arise secondarily on the slope of an old one.

A simple sink-hole is one without secondary sinks. Simple sink-holes agree in having the form of an inverted cone and are of three types. The first type has an opening at the apex of the cone leading to an underground passage.

The second type has this opening closed. This closure is inaugurated by rocks and earth caving in from the sides of the opening. Sediment composed of the fine clay from the sides is then deposited over this, and forms a very impermeable layer. This results in a more or less permanent pond.

The third type of simple sink-holes is like the second, except that a new opening to a subterranean passage has been formed on its sides a short distance above its lowest point. This new opening is formed from below, but the details of the process I could not determine. The result is a pond, which at its higher levels overflows into the new opening.

Open sink-holes are the rule in land covered with timber, because the erosion in such areas occurs slowly.

The plankton is derived from the simple sink-holes of the third type and compound sink-holes in which the lower sinks are open and the upper are closed, and in which the divides are low enough to allow the upper ones to overflow into the lower. The first type of simple sink-holes allows the water to flow so quickly into the underground channel, that organisms do not have time

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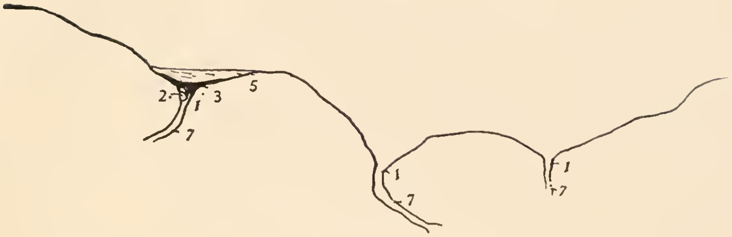
[1] The cave region is a plateau which has not yet developed surface streams. The surface of this region is drained by funnel-shaped depressions called "sink-holes."



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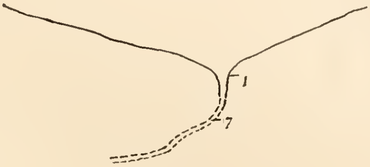

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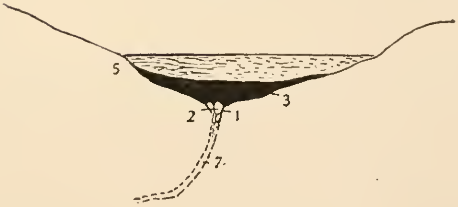

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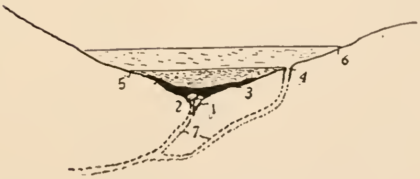

Diagrammatic section of compound and simple sink holes. 1, original opening; 2, obstruction; 3, impervious layer of clay; 4, second opening; 5, ordinary water-level; 6, flood water-level; 7, subterranean passage.

to develop. A few wind-blown eggs and cysts may be carried into the cave and develop there, but I think it extremely doubtful. The second type of simple sinks develops many organisms, but they are not admitted to the cave stream.

Temporal Distributions. — The temporal distribution of the plankton in this cave is puzzling and the causes of this distribution are so complex that their analysis is difficult.

Although qualitative methods only were used during the fall and winter, there was less plankton in early winter than in the autumn. From this it was tentatively concluded that the main outlines of the temporal distribution of the cave plankton coincide with that of lakes and rivers in temperate latitudes, in having spring and autumnal maxima, and summer and winter minima.

After a rain of 4.71 inches which fell on February 13 and 14, I was unable to detect any organisms in the catch. Possibly a few were present and were overlooked because of the great amount of silt.

In March a series of quantitative collections was begun and continued for six months. During this period, two maxima occurred. One was on May 14, and the other was on August 13. These were maxima both in number of individuals and in number of species. Each was preceded by a very heavy rain. Between these maxima there were no rains sufficient to affect the volume of the cave stream. The maximum of May was larger than that of August (see Table II).

The February flood was accompanied by a marked decrease, but those of May and August were accompanied by a sudden increase and followed by a gradual decrease.

From these facts it is safe to conclude that excessive rainfall influences the amount of plankton per gallon of water in the cave, but not always in the same way. Excessive rainfall affects the amount of plankton in three ways. (1) The pools in plankton producing sinks of the land surface over the cave overflow and the organisms contained in them are carried into the cave. (2) The plankton is diluted in the sink where it is produced and is further diluted in the cave by the water from sink-holes of the first type. (3) The stream level is raised and the current is increased This increase in the current causes many organisms to

TABLE II.

SHOWING INFLUENCE OF FLOOD UPON TEMPORAL DISTRIBUTION.

Date.	CLASS OR GENERA.					Rainfall in Inches for Week Ending
	Cyclops.	Nauplii of *Cyclops.*	Rotifera.	*Difflugia.*	*Bosmina.*	
March 24	85	63	28	5	0	.53
" 30	75	93	74	6	0	.89
April 7	44	50	42	6	1	1.26
" 14	29	20	12	6	1	1.13
" 21	14	12	15	6	3	.32
" 28	11	46	18	3	1	.31
May 7	count incomplete on account of large amount of silt.					5.46
" 14	410	319	717	21	1998	.00
" 26	85	47	24	30	32	.85
June 2	34	30	20	16	6	.00
" 9	10	10	5	4	5	.66
" 16	11	23	14	2	6	.17
" 23	22	55	2	7	0	.00
" 30	17	42	3	10	0	.20
July 7	9	31	1	4	1	.12
" 14	28	139	3	18	0	.00
" 21	21	92	3	2	0	.15
" 30	18	84	15	5	15	.85
Aug. 7	277	325	32	34	38	1.85

be carried through the cave that would succumb to the cave environment at ordinary stream levels. The first and third of these effects of excessive rainfall are therefore positive and the second negative.

The cycle of pond life in this region is not well known, but is being investigated. It is very probable that in its main outlines it resembles that of lakes in the same latitude.

The minimum of February was due to two things, the small number of organisms produced in the pond and the great dilution by rain water.

The increase in the amount of plankton after the heavy rains of May and August was due to the fact that the amount contributed was so great that the dilution was insufficient to reduce it to the amount per gallon previously present in the cave. The increase in the number of species was caused by the rapid current carrying through many of the forms that would have succumbed to the destructive influences of the cave environment at lower stages of the water. The subsequent gradual decrease in amount and number of species was due to the destructive cave environment.

The temporal distribution of the plankton in this cave depends upon: (1) the production of the plankton in the ponds, (2) the overflow of the ponds into the subterranean passages, (3) the dilution by rain water and interstratal seepage, (4) the rate of current in the cave stream, (5) the ability of the planktonts to live in the cave environment.

Local Distribution. — Observations taken at Dalton Cave and at Shawnee Cave indicate that the amount of plankton in the lower portion of the cave is less than in the upper portion, the amount at Shawnee varying from 40 per cent. to 80 per cent. of the amount at Upper Dalton. This is due, I think, principally to the destructive influence of the cave environment (see Table III.).

TABLE III.

Genus or Class.	Number per 200 gal. at Upper Dalton Cave.	Number per 200 gal. at Shawnee Cave.
Cyclops	59	41
Nauplii of *Cyclops*	89	64
Daphnia (immature)	0	1
Difflugia	11	3
Arcella	1	0
Œdogonium	1	0
Pandorina	2	0
Rotifera	2	4
Total	165	113

Feeding and Reproduction of the Plankton in the Cave. — The alimentary tracts of the crustacea always contained some food. In the alimentary tracts of *Cyclops* and *Bosmina* at ordinary stages of the cave stream, there was nearly as much food as in members of these genera taken above ground. In the lower stages of the stream the amount of food contained decreased.

Eggs were common in the egg sack of *Cyclops*, in the brood chambers of *Bosmina*, and upon *Anura cochlearis*. They were also observed in *Daphnia*, but the individuals of this genus were rare.

Nauplii of *Cyclops* were common throughout the year and the young of *Daphnia* and *Bosmina* were observed.

It is evident that some of the planktonts are able to continue their nutritive and reproductive processes under cave conditions, although this environment inhibits them. Whether these forms would be able to maintain themselves in a permanent pool cut off from the cave stream is conjectural, as no such pool exists in this cave.

Tha the algæ could not live in the total darkness of the cave is certain. The method was not adapted to the investigation of the bacteria and the infusoria.

Relation of the Cave Plankton to the Permanent Cave Fauna.— The plankton of this cave does not form nor can it become a part of the permanent fauna of the cave, because the current of the stream in its higher stages is powerful enough to carry out of the cave all forms that are not strong swimmers, or have not developed the habit of living under rocks or on the bottom. *Cæcidotea*, *Crangonyx* and *Cambarus* live on the bottom and under rocks. *Ambylopsis* is a fairly strong swimmer and when struck by a current goes to the bottom or under a rock. These animals are examples of forms whose habits prevent them from being carried out of the cave.

Banta has taken *Cyclops* from the stomach of *Amblyopsis spelæus.* This suggests that the plankton is a source of food for some of the permanent cave animals.

The organisms of the cave plankton are essentially pond forms and their presence in the cave is accidental. They do not migrate into the cave to colonize it.

Summary and Conclusions. — 1. The form of the cave is determined by the direction of the seams in the limestone, the obstructions, and a factor X, which is probably elevation.

2. The form of the cave thus determined results in a large amount of residual water in the cave.

3. This residual water causes the extreme flood temperature to occur twenty-four hours or more after the crest of the flood. It also causes the rate at which the plankton is carried through the cave to vary directly as the stream level varies.

4. Water temperature varies much more than the air temperature in the interior of the cave.

5. Air currents in this cave are caused by and their rate varies directly with the divergence of terranean and subterranean temperature.

6. The amount of plankton in the cave is relatively small.

7. The plankton is composed of epigean forms and is derived from ponds in such sink-holes as have an opening above their lowest points.

8. Temporal distribution of the plankton depends upon three principal factors, the production of the plankton in the pools, excessive rainfall, and the ability of the planktons to withstand cave conditions.

9. At ordinary stages of the stream more plankton occurs in the upper part of the cave than in the lower part.

10. Some of the planktonts feed and reproduce in the cave, but these processes are more or less inhibited.

11. The plankton is not a part of the permanent cave fauna but is essentially a pond fauna accidentally carried into the cave. It is either carried through the cave or perishes in it.

LIST OF SPECIES.[1]

Protozoa.

Arcella vulgaris Ehrenberg.
Difflugia globosa Dujardin.
D. pyriformis Perty.
D. lobostoma Leidy.
D. acuminata Ehrenberg.
Euglena sp.

Arthropoda.

Cyclops bicuspidatus Claus.
C. prasinus Fischer.
C. viridus Jurine.
C. serrulatus Fischer.
C. edax Forbes.
Canthocamptus sp.
Daphnia pulex DeGeer.
Ciriodaphnia consors Birge.
Bosmina bohemica Hellick. (?)
B. cornuta Jurine.
Cydorus sphæricus Mueller.

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[1] Diatoms were taken occasionally. The collections sometimes contained spiders which doubtless had fallen from the walls of the cave. One round worm and two segmented worms were observed during the year.

Pleuroxus hamatus Birge.
Cypris sp.
Campodea staphylinus (?) Westwood.
Larvæ of diptera and coleoptera rarely.

TROCHELMINTHES.

Asplanchna ebbesborni Hudson.
Triarthra longisetæ Ehrenberg.
Cathypna luna Ehrenberg.
Monastyla lunaris Ehrenberg.
M. bulla Gosse.
Branchionis militaris Ehrenberg.
B. bakeri Ehrenberg.
Noteus quadricornus Ehrenberg.
Anura cochlearis Gosse.
Notholca longispina Kellicott.

ALGÆ.

Œdogonium sp.
Cladophora sp.
Vaucheria sp.
Spirogyra sp.
Zygnema sp.
Closterium subcostatum Nord.
C. lanceolatum Kg.
Cosmarium sp.
Micrasterias sp.
Volvox globator Ehrenberg.
V. aureus Ehrenberg.
Pandorina morum Bory.
Pleurococcus sp.
Pediastrum boryanum Menegh.
Senedesmus sp.
Sphærocystis schrœteri Chod.
Nostoc minutissimum Kg.
Oscillatoria sp.

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Explanation of Map.

Shawnee Cave (the outlet). Sec. I., No. 1.
Closed chamber caused by collapse of roof at Sec. I., Nos. 2–3.
Cascade. Sec. I., No. 6.
Double passage. Sec. I., Nos. 7–8.
Old cross cave. Sec. I., Nos. 9–10.
New passages. Sec. I., Nos. 1–8 and 11–13.
Opening in roof leading to *upper older* levels of cave. Sec. I., No. 14.
"Big Room." Sec. I., Nos. 15, 16, 17, 18, 19, 20, 21, 22.
"Fallen Rock." Sec. I., No. 31.
Lower Twin Cave. Sec. I., No. 32.
Upper Twin Cave. Sec. I., No. 33.
Roof too low for passage of boat. Sec. I., No. 34.
Deepest water in cave, 10 feet 4 inches. Sec. I., No. 35.
Lower Dalton Cave. Sec. I., No. 36.
Upper Dalton Cave. Sec. I., No. 37.

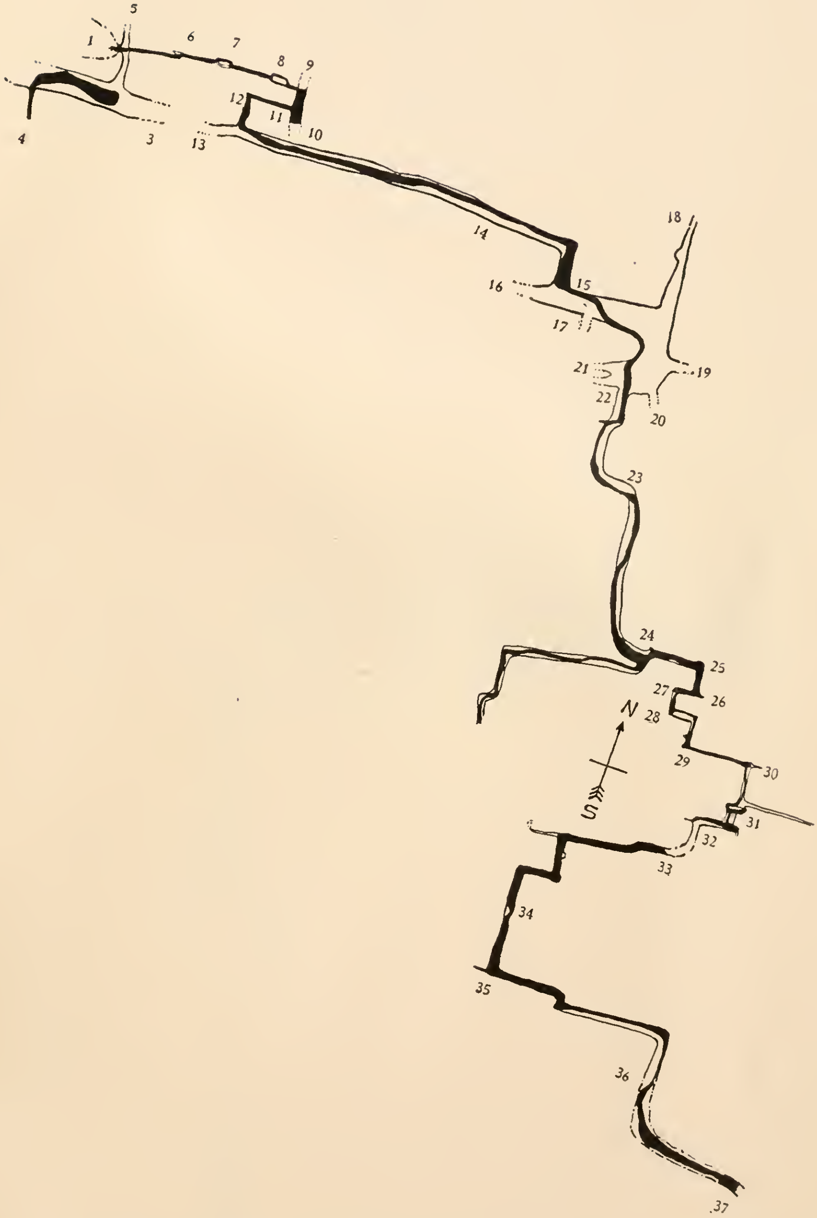
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FIG. 1. Map of Shawnee Cave, section 1, from Shawnee to Lower Dalton. Length 4,453 feet. Scale 200 feet to the inch.

Explanation of Map.

Upper Dalton Cave. Sec. II., No. 37.
"Cross bedding" in limestone. Sec. II., Nos. 46–47.
"Old passages." Sec. II,, Nos. 56–57.
Obstruction past which boat cannot be taken. Sec. II., No. 63.
End of exploration. Sec. II., No. 64.

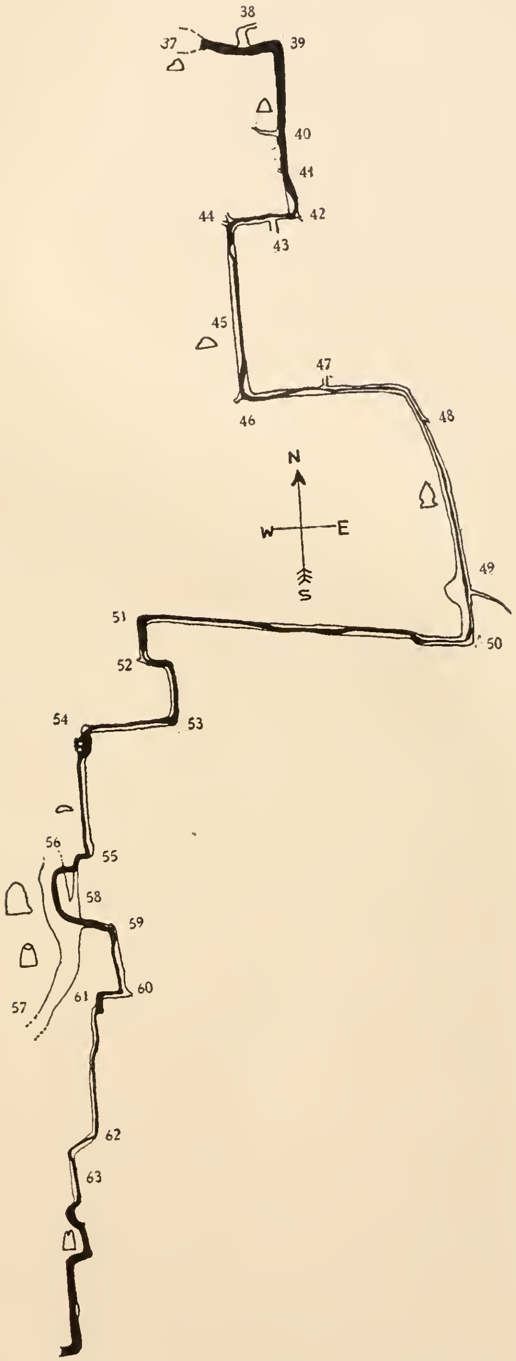
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FIG. 2. Map of Shawnee Cave, section 2, from Lower Dalton to unexplored part. Length 4,674 feet. Scale 200 feet to the inch.

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